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16. ABSTRACT

Methyl chloroform (MC) is a volatile chlorinated hydrocarbon used extensively as an industrial solvent and in consumer products. It has been detected in the ambient air of a variety of urban and non-urban areas of the United States. Normally, background levels are in the range of 0.1 to 0.2 ppb (0.54×10^{-3} mg/m^3). Levels in some urban areas have ranged up to 20 ppb (0.11 mg/m^3). It has been less frequently detected in water, generally at levels of 1 ppb or less. In certain instances involving contamination of groundwater, much higher levels have been reported.

The weight of available evidence obtained from both human and animal data suggest that long-term exposure to environmental levels of MC poses no serious health concern to the general population. No teratogenic potential has been demonstrated for MC in studies conducted to date in rodent species. Available data are inadequate for reaching firm conclusions about its mutagenic potential in humans. Because of the limited usefulness of the animal bioassays conducted to date, it is not possible to classify MC in regard to its carcinogenic potential in humans.

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Health Assessment Document for 1, 1, 1-Trichloroethane (Methyl Chloroform)

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. The health assessment was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of methyl chloroform as a hazardous air pollutant. However, the scope of this document has since been expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been critically evaluated and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses is placed in perspective with observed environmental levels.

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ABSTRACT

Methyl chloroform (MC) is a volatile chlorinated hydrocarbon used extensively as an industrial solvent and in consumer products. It has been detected in the ambient air of a variety of urban and non-urban areas of the United States. Normally, background levels are in the range of 0.1 to 0.2 ppb (0.54×10^{-3} to 1.1×10^{-3} mg/m^3). Levels in some urban areas have ranged up to 20 ppb (0.11 mg/m^3). It has been less frequently detected in water, generally at levels of 1 ppb or less. In certain instances involving contamination of groundwater, much higher levels have been reported.

The weight of available evidence obtained from both human and animal data suggest that long-term exposure to environmental levels of MC poses no serious health concern to the general population. No teratogenic potential has been demonstrated for MC in studies conducted to date in rodent species. Available data are inadequate for reaching firm conclusions about its mutagenic potential in humans. Because of the limited usefulness of the animal bioassays conducted to date, it is not possible to classify MC in regard to its carcinogenic potential in humans.

The no-observed-effect level for short-term exposure of humans is in the range of 350 to 500 ppm (1,890 to 2,700 mg/m^3).

1. EXECUTIVE SUMMARY

1,1,1-Trichloroethane (methyl chloroform, MC) is a volatile chlorinated hydrocarbon. Since its commercial introduction, it has been used increasingly as an industrial solvent and in consumer products, such as spot removers. Production of MC in the United States is estimated to have increased from 121,000 metric tons in 1970 to 315,000 in 1980. It is estimated that approximately 88 percent of annual production in the United States is released largely to the atmosphere through dispersive use. There are no known natural sources of emissions of MC.

Methyl chloroform has been detected in the ambient (natural environment) air of a variety of urban and non-urban areas of the United States and other regions of the world. Levels range from trace amounts in rural areas to about 20 parts per billion (ppb) or 0.108 mg/m^3 in some large urban centers. Normally background levels of MC are in the range of 0.1 to 0.2 ppb (0.54×10^{-3} to 1.08×10^{-3} mg/m^3). It has less frequently been detected in water. It is not soluble to any appreciable extent but has been monitored in some surface and drinking waters, generally at levels of 1 ppb or less. In certain instances involving contamination of groundwater, much higher levels have been reported.

In the troposphere, a region of the atmosphere extending from ground level to as high as 15 kilometers, MC is removed to a substantial extent through reaction with hydroxyl radicals. Based on current knowledge of its reaction kinetics, the lifetime of MC in the troposphere is in the range of 5 to 10 years. This time period allows a portion of the MC to be conveyed to the stratosphere where it, along with other compounds, may participate in ozone (O_3) destruction pathways. It has been hypothesized that MC and other compounds that add to the chlorine burden in the stratosphere may contribute to the effects of global O_3 depletion. If depletion occurs, it may result in a possible increased incidence of non-malignant forms of skin cancer due to increases in the amount of biologically-damaging radiation reaching the earth's surface. The extent to which past, current, and future emissions of MC contribute to O_3 depletion can only be realistically estimated by assessing the interrelationships between all the principal reactions involved in both the formation and destruction of atmospheric O_3. The extent and direction to which actual global O_3 levels have changed over the years is not estimable with available measurement methods.

Because MC is primarily an air contaminant, inhalation is the principal and most rapid route of exposure. It is estimated that an 8-hour exposure to the TWA* of 350 parts per million (ppm) or 1,890 mg/m^3 will result in about 2 grams of MC absorbed into the body of an average-sized 70 kg man. The total amount absorbed increased in direct proportion to inspired air concentrations and to the length of exposure and physical activity. Once body equilibrium or steady-state has been attained, no further uptake is possible. There is strong evidence that MC will partition selectively into lipid-tissues upon chronic or long-term exposure to even low ambient air concentrations, until steady-state is attained. Because of its lipophilic nature, MC is expected to cross membrane barriers in the body and diffuse into the brain and the colostrum of nursing mothers, as well as into the fetus during pregnancy. Unlike other chlorinated solvents such as trichloro- and tetrachloroethylene, MC is only metabolized in humans to a limited extent, about 6 percent or less of the total retained dose. Although metabolism of MC is affected by other chemicals and drugs, there is no evidence that it enhances its own metabolism. The primary route of elimination from the body is via the lungs, through which MC is exhaled in unchanged form along with a metabolite, carbon dioxide. The only identified urinary metabolites are trichloroethanol and trichloroacetic acid.

The likelihood of adverse health effects resulting from chronic exposure to the ambient air levels commonly encountered appears to be extremely low based on presently available data. It is estimated that a no-observed-effect-level (NOEL)** for short-term exposure of humans to MC is in the range of approximately 350 to 500 ppm (1,890 to 2,700 mg/m^3). This NOEL is many orders of magnitude higher than the highest levels of MC (20 ppb; 0.108 mg/m^3) measured in the ambient air of urban areas. Based upon available human data, the estimated relationship between acute effects and single short-term exposures is as follows:

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*TWA (Time Weighted Average): is defined as the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek to which nearly all workers may be exposed repeatedly, day after day, without adverse effect.

**NOEL: is defined as the lowest exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

100 ppm (540 mg/m^3)	Apparent odor threshold
350-500 ppm (1,890 - 2,700 mg/m^3)	Obvious odor, slight changes in perception
1,000 ppm (5,400 mg/m^3)	Disturbances of equilibrium
1,900 - 2,650 (10,260 -14,310 mg/m^3)	Lightheadedness, irritation of the throat
> 5,000 ppm (27,000 mg/m^3)	Onset of narcosis

In the range of the NOEL no significant abnormal blood chemistry or organ function decrements have been noted. The main health effects are symptoms of neurological dysfunction observed at higher exposure levels. These symptoms were qualitatively diagnosed by subjects' impaired performance of clinical-level cognitive and manual tasks. More extensive human and laboratory animal data would be needed before firm conclusions about adverse health responses to low-level exposures to MC could be drawn.

Similarly, MC has not demonstrated any teratogenic potential in the studies conducted to date in rodent species. Commercially available samples of MC are genotoxic to mouse hepatocytes and are weakly mutagenic in Salmonella under treatment conditions where sufficient exposure is ensured. The available data are inadequate, however, for reaching firm conclusions regarding the ability of MC to cause gene mutations in other organisms; however, the possibility that this substance, its associated stabilizing materials, or its metabolites may have mutagenic effects in humans has not been eliminated.

On the basis of animal bioassays performed to date and in the absence of epidemiological information, it is not possible to classify MC as to its carcinogenic potential in humans.

The weight of available evidence obtained from both human and animal data suggest that long-term exposure to environmental levels of MC poses no serious health concern to the general population. One must recognize, however, that as new information becomes available, further re-evaluation of the health consequences of exposure may become necessary.

RECOMMENDATIONS FOR FURTHER STUDIES

Although the available human and toxicity data indicate that ambient exposure to MC is not currently a human health concern, it is apparent that further investigation is needed in several areas. Areas in which incomplete information is available, and that should be considered in formulating research needs, are presented below. These research needs, however, are not listed below in relation to relative priorities.

1. Neurobehavioral toxicity. There have been few animal studies of the effects of MC on the nervous system and behavior. Most endpoints studied have been relatively insensitive. Further studies are warranted on more sensitive endpoints.
2. Teratogenicity and Reproductive Effects. There are no published three-generation animal studies via inhalation that adequately assess the teratogenic and reproductive effects potential of MC. Uncertainty about these biological endpoints in humans should serve as a stimulus for future research.
3. Mutagenicity. In order to determine conclusively the mutagenic potential of MC, pure MC should be tested in studies appropriately designed to ensure exposure of the indicator organism.
4. Carcinogenicity. In order to determine more effectively the human carcinogenic potential of MC, lifetime inhalation studies in appropriate rodent species should be undertaken.

2. INTRODUCTION

1,1,1-Trichloroethane (methyl chloroform, MC) belongs to a family of saturated chlorinated compounds widely used in industrial cold cleaning and vapor degreasing operations, in the synthesis of vinylidene chloride, and in consumer products. This document provides an evaluation of the health effects of MC and a review of the relevant available scientific literature. In order to provide a perspective in evaluating health effects associated with MC, this document contains background chapters relating to analytical methodologies, production, sources and emissions, and ambient[a] air concentrations.

Methyl chloroform is released into the ambient air as a result of evaporation during its production, storage, and manufacturing or during general consumer use. It is not known to be derived from natural sources. In recent years, attention has been focused upon the possible role of MC in multiple perturbations of stratospheric ozone. It has been hypothesized that stratospheric ozone depletion may indirectly result in increased ultraviolet radiation at the earth's surface, thereby contributing to an increased incidence of human skin cancer. MC is one of the many atmospheric pollutants with a reactivity sufficiently low to allow it to be transported to the stratosphere; its tropospheric lifetime is estimated to be 5 to 10 years. Levels of MC in ambient air sampled around the nation reflect both efforts in recent years to control emissions of this halocarbon and transport and transformation processes in the atmosphere affecting its distribution.

The published scientific literature on MC is limited in reference to its effects on humans. Because of the diverse uses of MC and the difficulty of identifying large cohort populations, there are relatively few epidemiological studies. What is known about the effects of MC has been primarily derived from studies involving individuals exposed occupationally or accidentally to MC. In such exposures, the concentrations of MC associated with adverse effects to human health were either unknown or far in excess of the concentrations measured in ambient air. Controlled exposure studies of humans have been carried out at TWA[b] concentrations (350 ppm; 1,890 mg/m^3) and have been directed toward elucidation of subjective responses, CNS effects, and the pharmacokinetic parameters of MC exposure. These studies have established that vapor inhalation is the principal route by which MC enters into the body. It is widely distributed into all organ systems and is metabolized to a limited extent. MC is eliminated from the body primarily as the parent compound via the lungs, but metabolites are excreted mostly in the urine. Epidemiological studies provide some information about the impact of MC on human health, but it is necessary to rely on animal studies to assess any indications of potentially harmful effects for chronic low-level exposures. These studies are reviewed in chapters 4 and 5.

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[a] Ambient is used in this and other documents of this nature to refer to the environment and should not be construed to include indoor, and occupational settings.

[b] TWA (Time Weight Average): is defined as the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek to which nearly all workers may be exposed repeatedly, day after day, without adverse effect.

Of chief concern regarding the potential impact of MC on human health are: (1) narcosis effects associated with acute high-level exposures and (2) any mutagenic or carcinogenic effects potentially associated with chronic low-level exposures. This document reviews evidence regarding acute narcosis effects and, also, summarizes available mutagenicity evidence and peer-reviewed results of animal bioassay studies that relate to the carcinogenic potential of MC. A definitive evaluation of the carcinogenicity of MC is being deferred until the results of a recently-completed National Toxicology Program bioassay in rats and mice undergo complete peer review.

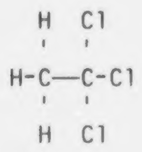
Permissible levels of MC in the working environment have been established in various countries. The U.S. Occupational Safety and Health Administration (OSHA) health standard requires that a worker's exposure to MC at no time exceed a TWA of 350 ppm (1,890 mg/m^3) in the workplace air in any work shift of a 40-hr week.

3. GENERAL BACKGROUND INFORMATION

3.1 CHEMICAL AND PHYSICAL PROPERTIES, ANALYTICAL METHODOLOGY

3.1.1 Chemical and Physical Properties

1,1,1-Trichloroethane (CH_3CCl_3), also called methyl chloroform (MC), is a colorless nonflammable liquid which has a characteristic odor. Its line formula is:



$$\begin{array}{cccc} & H & & Cl \\ & | & & | \\ H- & C & — & C-Cl \\ & | & & | \\ & H & & Cl \end{array}$$

Chemical Formula $C_2H_3Cl_3$

Chemical Abstracts Service Registry Number 71-55-6

Synonyms include:

Ethane, 1,1,1-Trichloro
Methyl Chloroform
Methyltrichloromethane
Trichloroethane
1,1,1-Trichloroethane
α-Trichloroethane

Table 3-1 shows some of its important chemical and physical properties.

TABLE 3-1. PHYSICAL PROPERTIES OF 1,1,1-TRICHLOROETHANE

Solubility in water @ 25°C	0.44 gm/100gm
Boiling point @ 760 torr	74°C
Vapor pressure @ 20°C	100 torr
Vapor density (air = 1)	4.6
Molecular weight	133.41
1 ppm	5.4 mg/m^3

In the atmosphere, MC is subject to free radical attack. Reaction with hydroxyl radicals is the principal way in which MC is scavenged from the atmosphere. Photooxidation products of MC identified via laboratory experimentation include hydrogen chloride, carbon oxides, phosgene, and acetyl chloride (Spence and Hanst, 1978; Christiansen et al., 1972). The tropospheric photooxidation products are numerous and rapidly undergo further reactions

(U.S. Environmental Protection Agency, 1975). Data discussed in Section 3.3 indicate that MC is relatively stable in the troposphere and that significant amounts are conveyed to the stratosphere.

The volatilization of MC from water in laboratory studies was investigated by Dilling and coworkers (Dilling et al., 1975; Dilling, 1977). A half-life of from 17 to 30 minutes was observed for MC dissolved in water in a laboratory beaker. These investigators determined that degradation proceeded with a 6-month half-life and that the rate of degradation was not increased significantly upon exposure to sunlight. In the preliminary studies of Wood et al. (1981), degradation of MC was observed under anaerobic conditions in the presence of natural water samples. Sediment water samples obtained from the Florida everglades were placed in sealed serum bottles and spiked with 200 μg of MC. Degradation was observed to proceed via 1,1-dichloroethane; this intermediate suggests the mechanism involves reductive dehalogenation of MC. Under these conditions, the observed half-life of MC was about 16 days. Degradation of MC was not observed in autoclaved controls.

Anhydrous MC is generally noncorrosive, but in the presence of water it can react to form hydrochloric acid, which is corrosive to metals (Keil, 1979). The addition of epoxides can neutralize the generated acid (Keil, 1979). Anhydrous MC, when heated to 360° to 440°C, decomposes to 1,1-dichloroethylene and hydrogen chloride. When MC is heated in the presence of water at temperatures between 75° and 160°C, it decomposes to acetyl chloride, acetic acid, and acetic anhydride upon contact with metallic chlorides or sulfuric acid. Noweir et al. (1972) have observed that when MC comes in contact with iron, copper, zinc, or aluminum at elevated temperatures, a small amount of phosgene is produced. During welding operations, the amount of hydrogen chloride produced, in contrast to phosgene, may be sufficient to provide adequate warning to minimize exposure (Rinzema and Silverstein, 1973).

Bonse and Henschler (1976) have considered the general chemical reactivity of MC. By virtue of the electron-inductive effects of the chlorine substituents, destabilization occurs at the C-C bond. Radical reaction mechanisms would be favored as a result of the delocalization of the unpaired electron drawn from the C-C bond.

3.1.2 Analytical Methodology

To detect very low levels of methyl chloroform in ambient air, sophisticated analytical techniques have been employed. The two most generally useful methods for detection and analysis of MC have been gas chromatography with electron capture detection (GC-ECD) and gas chromatography-mass spectrometry (GC-MS). Both systems have a lower limit of detection on the order of a few parts per trillion (ppt). The utility of GC-ECD over GC-MS is that it can be used in the field to provide nearly continuous measurements through intermittent sampling (every 15 to 20 minutes).

In a comparison between GC-ECD and GC-MS, Cronn et al. (1976) judged GC-ECD to be superior in precision. Standards for four halocarbon compounds, measured by GC-ECD, had coefficients of variation ranging from 0.5 to 3.5 percent. Of 11 halocarbon compounds measured by GC-MS, coefficients of variation ranged from 4 to 19 percent. Temperature programming or isothermal operation of the gas chromatograph yielded comparable results (Cronn et al., 1977a). The precision with temperature-programming was 4.3 percent versus 3.5 percent with isothermal operation for MC.

A close agreement between the levels of MC and other halocarbons detected by both GC-ECD and GC-MS (on the same ambient air samples) was obtained by Russell and Shadoff (1977). A difference of 10 ppt was reported between the systems. Air samples were sorbed onto Porapak N porous polymer and desorbed onto the column by heating the collection tube rapidly to 200°C. Ambient air mixing ratios measured by this method were in the range of 90 to 110 ppt.

The electron capture detector, as well as the mass spectrometer in the selective ion monitoring mode (Cronn and Harsch, 1979a; Pellizzari, 1974), is specific in the detection and quantitation of many halogenated hydrocarbons; nonhalogenated hydrocarbons cannot be detected. Thus, a high background level of hydrocarbons in ambient air samples does not preclude analysis of trace quantities of MC. In the electron capture detector, MC is ionized by primary electrons released from an internal radioactive source. The net result is removal of electrons from the gaseous mixture with substitution by negative ions of greater mass. The measured effect is a net decrease in ion current because the mobility of the ions is less than that of the electrons.

Samples of tropospheric air were analyzed by Cronn and coworkers (1977b) both by preconcentrating the samples according to the method of Rasmussen et al. (1977) and by direct injection. The detection limit was 3 ppt ($16.2 \times 10^{-6} mg/m^3$). The precision of the method using preconcentration was ±4 percent. The internal consistency between direct GC-ECD analysis of MC and using the preconcentration method was reported to be good (Cronn et al., 1977b).

A detection limit of 2 ppt ($10.8 \times 10^{-6} mg/m^3$) for MC was achieved by Harsch and Cronn (1978) with a low pressure sample-transfer technique. Precision was ±7 percent with the Rasmussen et al. (1977) preconcentration technique. Calibration was accomplished by use of secondary standards that were compared to static dilutions of pure and commercially prepared mixtures of MC (10 ppm; 54 mg/m^3) in helium. Robinson (1978) reported a detection limit of 6 ppt with a precision of ±4.2 percent with secondary standards in a system using dual GC-ECD.

Pellizzari and Bunch (1979) reported an estimated detection limit of 12.45 ppt (66×10^{-6} mg/m^3) using high resolution gas chromatography/mass spectrometry. The detection limit was calculated on the basis of the breakthrough volume for 2.2 grams of Tenax GC, at 21°C. The accuracy of analysis was reported at ± 30 percent. Similar results were also reported by Krost et al.(1982). Sources of error are discussed in section 3.1.2.1.

The relatively high volatility of MC was used to advantage by Piet et al. (1978) in measuring the content of MC in water samples. A direct headspace method was used with MC volatilizing into the air above the surface of the water sample in a sealed container. A GC-ECD system was used for separation and quantitation. The systematic error of the method was reported to be less than 5 percent. The detection limit for MC was 0.05 μg per liter. The OV 225 capillary GC column provided good separation of MC from CCl_4.

Otson and Williams (1982) have described a modified purge and trap technique for evaluation of volatile organic pollutants in water. The detection limits reported for MC was 0.5 μg/l, using either flame ionization detection or electron capture. Tenax GC was used as packing for the combined trap/chromatographic column. Purging efficiency was close to 100 percent.

Lionel et al. (1981) provided additional evidence that aeration can be used successfully to purge compounds such as MC from water. Experimental techniques suggest that at appropriate air flow, over 90 percent of MC can be purged from water.

The purging efficiency from samples of sediment and fish tissue spiked with MC was investigated by Hiatt (1981). Samples were sonicated, frozen, and then subjected to three analytical methods: vacuum extraction, direct purge and trap of diluted sample, and modified purge and trap with thermal desorption. For MC, vacuum extraction appeared to result in the highest recovery. These preliminary data do suggest that vacuum extraction has application to environmental analysis of similar samples.

Development of analytical procedures for quantitation of MC and other compounds from biological media such as mother's milk has been evaluated by Pellizzari et al. (1982). All volatile compounds were recovered by warming the milk and purging with helium. Vapors were then trapped on a Tenax cartridge, followed by thermal desorption and analysis by GC-MS.

3.1.2.1 Sampling and Sources of Error--The National Academy of Sciences (1978) has reviewed common approaches used to sample ambient air for trace gas analysis. These approaches include:

1. Ambient pressure samples. An evacuated chamber is opened and allowed to fill until it has reached ambient pressure at the sampling location. If filling is conducted at high altitude, contamination of the low pressure sample is likely when samples are returned to ground level.
2. Pump pressure samples. A mechanical pump is used to fill a stainless steel or glass container to a positive pressure relative to the surrounding atmosphere.
3. Adsorption on molecular sieves, activated charcoal, or other materials. Sorbents that have been used to collect MC include Porapak Q, Tenax GC, and Chromosorbs 101, 102, and 103.
4. Cryogenic sampling. Air samples are transferred to a loop held at the temperature of liquid oxygen. Components, including MC, are concentrated by freezeout while other gases (oxygen, nitrogen) pass through.

In the analysis of whole air samples of compounds such as MC, which are present in ambient air in the ppt range, small uncontrolled errors in the system could result in inaccurate results. Errors in the analysis can occur during sampling, in the gas chromatograph, in the EC detector, and in calibration of the instruments.

Rasmussen and Khalil (1981a) reported that, in an interlaboratory comparison of two samples of MC in ambient air, a majority of the 19 participating laboratories reported values lower than those determined by the authors. It was concluded that common standards are needed if atmospheric measurements from different laboratories are to be pooled.

Singh and coworkers (1978a) used electrochemically-polished stainless steel sampling vessels. The sampling vessels were flushed with ultra-pure helium until background contamination was reduced to less than 2 to 3 percent of the expected background concentration of a given trace constituent. Cronn and coworkers similarly employed electropolished stainless steel containers whose interior surfaces had been passivated by electropolishing (Cronn and Robinson, 1979; Cronn et al., 1976; 1977).

Harsch and Cronn (1978) have evaluated sample transfer techniques. Most analytical procedures involve the collection of pressurized air samples. Sample transfer under positive pressure minimizes serious contamination possibilities posed by laboratory air containing many times the ambient mixing ratios of most halocarbons. However, collection of low pressure samples, such as stratospheric air samples, is a specialized situation in which careful precautions against sample contamination are necessary. A technique applicable to collection of whole air samples at altitudes up to 21 kilometers showed a 7 percent standard deviation when the MC mixing ratio was 105 ppt (Harsch and Cronn, 1978). Electropolished sampling bottles were evacuated to 30 microns prior to sample collection. Transfer of contents to a freezeout loop (Rasmussen et al., 1977) immersed in liquid O_2 was made by vacuum assist. Preconcentration was followed by injection onto a temperature-programmed column. The detection limit for MC with the GC-ECD procedure was 2 ppt (10.8×10^{-6} mg/m^3). To minimize the number of potential sources of leaks, system components were silver soldered. The technique was applicable when gross halocarbon levels were not present in laboratory air. However, it was noted that if laboratory air contained ppb levels of halocarbons, high-boiling halocarbons were adsorbed onto the surfaces of the fittings of the sampling containers and associated plumbing during times when the system was exposed to laboratory air.

The EC detector can be a source of error because water vapor and oxygen may cause reduced sensitivity for certain halocarbons. Lillian and Singh (1974) used an ascarite trap between the GC column and the detector to absorb sample moisture. Carrier gas for the GC is purified by passage through traps containing activated charcoal, anhydrous calcium sulfate, and an ascarite or molecular sieve (Cronn, 1980a). Oxygen contamination in the detector can be minimized by preconcentration of air samples on porous polymers (Russell and Shadoff, 1977). Significant oxygen contamination is often caused by fitting leaks in the GC rather than by carrier gas contamination.

A freezeout concentration method was employed by Rasmussen et al. (1977) to determine atmospheric levels of MC in the presence of other trace vapors. The detection limit of MC was reported to be 0.8 ppt (4.3×10^{-6} mg/m^3) for

500 ml aliquots of ambient air when measured by GC-ECD. Precision was 4.3 percent. Standards were prepared by static dilutions in helium. During the procedure, the oven of the GC was cooled to -10°C. When freezeout was complete, the loop containing the concentrated air sample was immersed in heated water and the carrier gas swept the contents of the sample loop onto the column.

Pellizzari and Bunch (1979) reported the use of Tenax GC, a porous polymer based on 2,6-diphenyl-p-phenylene oxide, to absorb MC from ambient air. Recovery was made by thermal desorption and helium purging into a freezeout trap. Included among the inherent analytical errors were (1) the ability to accurately determine the breakthrough volume, (2) the percent recovery from the sampling cartridge after a period of storage, and (3) the reproducibility of thermal desorption from the cartridge and its introduction into the analytical system. To minimize loss of sample, cartridge samplers should be enclosed in cartridge holders and placed in a second container that can be sealed, protected from light, and stored at 0°C. In an analytical system using Tenax GC as the absorbent, Pellizzari and coworkers (Krost et al., 1982) reported a detection limit for MC of 12.4 ppt (6.7×10^{-5} mg/m^3). Analysis of the breakthrough volumes at various temperatures suggests that Tenax GC is not a particularly effective absorbent for MC. Singh et al. (1982) have cautioned that absorption of MC on Tenax may not reliably reflect ambient air levels since measurements by some investigators have been reported as less than background (100 to 200 ppt; 0.54×10^{-3} to 10.8×10^{-3} mg/m^3).

3.1.2.2 Calibration--In general, calibration of the instrumentation for GC-ECD analysis of methyl chloroform has involved static, multiple dilutions of pure material in the ppm range to ppt levels (Cronn et al., 1976; 1977a,b; Harsch and Cronn, 1978; Lillian and Singh, 1974; Singh et al., 1977a). Singh et al. (1977b) have cautioned that, in some cases, multiple dilutions of materials to ppt levels are tedious and prone to inaccuracies. Calibration problems were cited by The World Meteorological Organization (1982) as a factor responsible for the discrepancies in tropospheric measurements by different investigators. In order to overcome the difficulties in generating low-ppb primary standards of MC, Singh et al., (1981) has reported that permeation tubes offer the most accurate means. Permeation tubes were conditioned for at least two weeks and, during standard generation, were kept in holders maintained at 70 ± 0.1°C. Error in the permeation rate (980 mg/min) was ± 15 percent. A reproducible means of generating low-ppb primary standards was judged essential since there are considerable difficulties in storing long-term secondary standards. Various investigators have used GC-ECD systems to quantitate MC in ambient air samples (Cronn et al., 1976; 1977; Lillian and Singh, 1974; Rasmussen et al., 1977; Singh et al., 1977c). An interlaboratory calibration (Cronn et al., 1976) of six real ambient air samples gave a precision (percent standard deviation) of 11.4 ppt. A similar comparison calibration was reported by Singh et al., (1981). An overall accuracy of ± 15 percent was reported for the preparation of secondary standards (Singh et al, 1982).

Rasmussen and Khalil (1981a) reported that primary standards of MC (~ 139 and 52 ppt) remained stable for up to about one year, even though the pressure in the master tanks had decreased significantly. More recently, Rasmussen and Khalil (1982) have found MC to be stable up to 2 years in internally passivated (SUMMA®) stainless steel flasks.

Because the ionization efficiency of MC in the EC detector is only 20 percent, use of dual EC detectors in series have been used to achieve higher accuracy (Lillian and Singh, 1974). The dual detection system, as employed by Singh et al. (1977c), enables MC to be quantitated coulometrically according to the following equation:

$$\text{Coulombs} = 96{,}500\ pW;$$

where

p = ionization efficiency

and W = moles of compound

Determination of the ionization efficiency can be made by use of the following expression which relates p to the signals (X_1 and X_2) of two identical EC detectors in series:

$$p = 1 - \frac{X_2}{X_1}$$

$$X_1 = 96{,}500\ pW$$

$$X_2 = p\ (96{,}500W - 96{,}500\ pW)$$

The ionization efficiency should be determined for the operating conditions and once established, can be used in the coulometric calibration.

The accuracy of the GC-ECD approach has been reported by Singh (1977a) to be better than 10 percent for compounds such as MC that have ambient air mixing ratios of 20 ppt (0.108×10^{-3} mg/m^3) or greater. An overall system accuracy of ± 10 percent was reported by Singh et al. (1977a) in analyses of MC in ambient air samples. Two gas chromatographs, each equipped with two EC detectors, were employed. An ascarite trap was placed between the GC column and the EC detector to prevent moisture interference. Precision was reported to be better than 5 percent and instruments were calibrated using multiple dilutions of pure material. Singh et al., (1981) recently reported linearity of dual frequency-modulated ECD's over a wide concentration range for MC, down to low-ppt levels.

3.1.2.3 Standard Methods--The analytical method S335, suggested by NIOSH for organic solvents in air, utilizes adsorption on charcoal followed by desorption with carbon disulfide. The resulting effluent is analyzed by gas chromatography. This method was recommended for the range 96 to 405 ppm (518 to 2,187 mg/m^3). Interferences are minimal and those that do occur can be eliminated by altering chromatographic conditions. However, one disadvantage is that the charcoal may suffer breakthrough, thus limiting the amount of air that can be sampled. This can be predicted, however, and multiple tubes can be used. Tubes could also be replaced at predetermined frequencies after shorter sampling periods to avoid breakthrough.

3.2 PRODUCTION, USE, AND EMISSIONS

Methyl chloroform is principally used for the cold cleaning and vapor degreasing of fabricated metal parts. Because of its volatility and dispersive use pattern, much of the MC produced worldwide is emitted into the atmosphere. There are no identified natural sources of emissions. Once in the atmosphere, MC is subject to atmospheric transport and transformation. To better assess the effects of present and future emissions of MC on human health this section profiles MC production, usage, and emissions.

3.2.1 Production

Methyl chloroform is produced by several manufacturing processes (Jordan, 1979; Lowenheim and Moran, 1975; U.S. Environmental Protection Agency, 1979b):

1. Hydrochlorination of 1,1-dichloroethylene (vinylidene chloride)

$$CH_2 = CCl_2 + HCl \xrightarrow{FeCl3} CH_3CCl_3$$

2. Hydrochlorination of vinyl chloride

$$CH_2 = CHCl + HCl \xrightarrow{FeCl_3} CH_3CHCl_2$$

$$CH_3CHCl_2 \xrightarrow[Cl_2]{400°C} CH_3CCl_3 + HCl$$

3. Chlorination of ethane

$$CH_3CH_3 + 3Cl_2 \xrightarrow{370° \text{ to } 480°C} CH_3CCl_3 + 3HCl + \text{byproducts}$$

In order to prevent catalyst deactivation, hydrochlorination reactions should be anhydrous (Jorden, 1979). Temperature control of thermal reactors for chlorination must be maintained to ensure MC stability (Jordan, 1979). In reaction (3) above, ethyl chloride and vinylidene chloride are byproducts. Increased yields of MC can be obtained through recycling of these byproducts.

In 1975, over 60 percent of the U.S. production of MC was derived from the hydrochlorination of vinyl chloride; derivation from vinylidene chloride accounted for 30 percent (Lowenheim and Moran, 1975).

In 1977, the U.S. production figure for MC was 288,565 metric tons (U.S. Environmental Protection Agency, 1979b; U.S. International Trade Commission, 1977). The major producers and their production capacities for 1977 are shown in Table 3-2. Production of MC in the United States for 1980 was reported as 314,668 metric tons (U.S. International Trade Commission, 1980). Thus, over the 1977 to 1980 period, production increased at an annual rate of about 3 percent. Quoting production estimates supplied by Dow Chemical U.S.A., Singh and coworkers (1979a) reported that global production of the chlorocarbon had been increasing at an annual rate of 12 percent. Estimates did not take into account production in the Soviet Union and other eastern European countries. The Dow Chemical estimate for global production in 1977 was 470,000 metric tons. The annual production growth rate through 1977 was reported to be 7 to 10 percent by trade sources (Chemical and Engineering News, 1979). Future growth in production depends on the status of other chlorinated solvents under regulation for which MC may be substituted and upon economic conditions.

TABLE 3-2. MAJOR PRODUCERS OF METHYL CHLOROFORM. (Cogswell, 1978)

Organization	1978	Capacity (Metric Tons) 1982*
Dow Chemical, Freeport, TX	204,000	204,500
PPG Industries, Inc., Lake Charles, LA	79,000	159,000
Vulcan Materials Co., Geismar, LA	29,000	90,000

*These capacity estimates were provided in the 27 September 1982 Chemical Marketing Reporter and included production units on standby.

3.2.2 Usage

In 1975, cold cleaning and vapor degreasing operations accounted for 75 percent of the total end use of MC. Approximately 12 percent was used in the synthesis of vinylidene chloride (Lowenheim and Moran, 1975). Use in metal cleaning operations was reported to total 67 percent of 1977 production (Cogswell, 1978).

MC is also used as a solvent in adhesive formulations, as a spot remover, as a film cleaner, and as an additive in metal cutting oils (Jordan, 1979; Keil, 1979; Lowenheim and Moran, 1975). Most commercial formulations of MC are stabilized in order to: (1) prevent or retard oxidation, (2) chelate metal ions, (3) scavenge HCl, and (4) passivate metal surfaces (Jordan, 1979). Vapor degreasing grades of MC contain 3 to 7 percent (w/w) stabilizers and additives (Jordan, 1979).

The most commonly used commercial stabilizers are: 1,4 dioxane, 1,3-dioxolane, butylene oxide, methylethylketone, isobutylalcohol, nitromethane, and nitroethane (Torkelson, 1982).

3.2.3 Emissions

Emissions of MC arise during primary and end product production, during dispersive use applications, from storage containers, and during disposal of waste materials.

Singh et al. (1979b) have estimated global emissions in 1977 at 95 percent of the 300,000 metric tons produced. Lovelock (1977a) estimated that 500,000 metric tons were released globally in 1975. The estimate of the global emissions rate in 1976 by the National Research Council (1979a) was about 439,000 metric tons. The WMO (World Meteorological Organization, 1982), using data reported by Neely and Agin (U.S. E.P.A., 1980), estimated current global annual releases at 476,000 metric tons. According to McCarthy (1975), almost all the MC produced is eventually released to the atmosphere.

Recent estimates in a report prepared for the U.S. Environmental Protection Agency, Office of Toxic Substances, placed 1978 nationwide emission losses to air at 214,000 metric tons (Katz et al., 1980). A slightly higher rate (245,000 metric tons) for nationwide emissions from all sources for 1978 was reported by Anderson et al. (1980) in a study prepared for the U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards. Emission estimates by source category are shown in Table 3-3.

TABLE 3-3. 1978 EMISSION LOSSES TO AIR

Source	1978 emission losses to air in million pounds (kkg) and percentage of total released to air		
Production			
1. From Vinyl Chloride	0.21	(95)	0.04%
2. From Vinylidene Chloride	0.15	(67)	0.03%
3. From Ethane	0.03	(14)	0.01%
Metal Cleaning	351.70	(159,500)	75%
Aerosols	39.90	(18,100)	8%
Adhesives	38.37	(17,400)	8%
Textiles	6.44	(2,920)	1%
Paints	10.91	(4,950)	2%
Inks	6.13	(2,780)	1%
Drain Cleaners	0.61	(278)	0.1%
Pharmaceuticals	0.27	(124)	0.06%
Film Cleaning	0.48	(218)	0.1%
Leather Tanning	0.23	(104)	0.05%
Catalyst Preparation	0.06	(28)	0.01%
Miscellaneous	14.82	(16,730)	3%
Total released to the air*	470	(213,300)	(100%)

*An additional 63 million pounds (28,602 kkg) are released to the environment via solid waste and water.

Adapted from Katz et al. (1980).

Based on annual measurements of global MC since 1975, Rasmussen et al. (1981a) and Khalil and Rasmussen (1981) have determined that emissions of MC since 1974 have increased at an exponential rate of 8 percent per year. This is somewhat less than half the previous rate. The average rate of accumulation since 1975 was calculated at 12.4 ± 1.5 percent per year.

3.3. ATMOSPHERIC TRANSPORT, TRANSFORMATION AND FATE

The relation of ambient air mixing ratios of MC to the likelihood of health effects in humans is influenced by many theoretical processes that may occur in the troposphere and stratosphere. Such processes include: transformation of MC into other potentially harmful atmospheric components; stability of such components; urban transport; tropospheric chemical reactivity; and diffusion into the stratosphere where MC participates in ozone (O_3) perturbation reactions.

3.3.1 Tropospheric Removal Mechanisms and Residence Time

Prior to 1977, it was commonly believed that the residence time (i.e. the average time between release to and removal from the atmosphere) was in the range of 1 to 2 years (National Research Council, 1976). This residence time was derived from estimates of globally averaged hydroxyl radical (OH) levels in the range of 10×10^5 to 30×10^5 molecules cm^{-3}. Reliable estimates of globally averaged MC levels were not available. It is important to note that reaction with OH is the principal tropospheric removal mechanism by which many compounds, including MC, are scavenged from the troposphere.

More recent modeling estimates of the loading of the troposphere by MC have led to refined estimates of OH mixing ratios and thus, suggest that the residence time of MC is more likely to be in the range of 5 to 10 years. The World Meteorological Organization (1982) recently reviewed the data base and found that, given the uncertainties in release rates and absolute concentrations of MC as discussed by Logan et al. (1981), the current mixing ratios observed for MC appear to be consistent with a lifetime of 5 to 10 years and with a globally averaged OH mixing ratio of about 7×10^5 molecules cm^{-3}. This estimate was, in part, based on the redeterminations of the rate constant for reaction of OH with MC by Kurylo et al. (1979) and Jeong and Kaufman (1979). The lack of knowledge concerning MC emissions from Eastern Bloc countries was also a factor that had been considered. Since OH distribution has only been estimated through the use of models, estimates for the atmospheric lifetime of MC should be viewed cautiously.

Support for this estimate by the World Meteorological Organization can be seen in the data of Rasmussen and Khalil (1981b). These investigators reported that tropospheric measurements of MC made at six locations in the Northern and Southern hemispheres, between January 1979 and June 1980, were consistent with a lifetime in the range of 6 to 10 years. Average concentrations over both hemispheres were calculated from latitudinal profiles.

Using reaction rates current for 1981, Logan et al. (1981) calculated a global lifetime of 5 years, by modeling MC mixing ratios over time and latitude and with altitude profiles of OH averaged over an annual cycle. The model results agree with those of Campbell (U.S. Environmental Protection Agency, 1980) in that MC removal is particularly sensitive to OH levels in the tropics, a region that accounts for about 70 percent of the global sink. Approximately 50 percent of the global sink is calculated over the tropical ocean. It was emphasized that one-dimensional models are inappropriate for calculating the lifetime of a compound such as MC. The model of Logan et al. (1981) calculated a mean OH level in the northern hemisphere of 1×10^6 molecules cm^{-3}. This is somewhat higher than the earlier estimates (2.5×10^5 per cm^3) calculated by Crutzen and Fishman (1977).

Derwent and Eggleton (1981), using a two-dimensional model, calculated a global lifetime of 4.8 years. Derwent (1982) has recently evaluated global, hemispheric, and one-dimensional models in comparison to a two-dimensional model. This report discusses the limitations and strengths of the various types of models used to estimate residence time and thus, the inherent differences between models in relation to estimates of stratospheric ozone changes. Methyl chloroform was one of three halocarbons chosen for the evaluation. It was concluded that the simpler model, with averaged chemical reaction rates and parameterization of transport processes, tend to under- or overestimate OH oxidation thus leading to errors in calculation of residence time. With respect to MC, however, both one- and two-dimensional models are in agreement with respect to atmospheric lifetime, 4.3 and 4.8 years, respectively. In contrast, hemispheric and global models yielded a lifetime of about 9 years.

Singh and coworkers (1979b) found that field measurements support a 6 to 8 year global average residence time. Earlier, Singh et al. (1977a, 1977b) computed a global average residence time between 8 and 11 years, based upon the observed tropospheric distribution of MC and the rate of reaction with OH accepted at the time. Estimates from a number of other investigators, using different approaches, are consistent with a 5 to 12 year range (Altshuller, 1980; Chang and Penner, 1978; Lovelock, 1977b).

In conclusion, the estimates of the atmospheric lifetime of MC, as determined by reaction with OH, should be viewed cautiously given the limitations and uncertainties inherent in the various models. However, unless new information is forthcoming, the estimate of 5 to 10 years is consistent with the state-of-the-art knowledge of the troposphere.

3.3.2 Impact Upon the Ozone Layer

Current understanding of stratospheric science suggests that several substances produced by human activities may affect stratospheric O_3. A major goal of atmospheric scientists is to determine the net effect of all these substances on O_3 simultaneously.

The National Research Council (1982), drawing upon the data base developed by the World Meteorological Organization (WMO, 1982), recognizes MC to have the potential to perturb stratospheric O_3 levels. This potential was not expressed in quantitative terms as had been done for the two principal chlorofluorocarbons believed to be the most important, namely, dichlorofluoromethane and trichlorofluormethane (CF_2Cl_2 and $CFCl_3$). The Council has stated that "The abundance of ozone in the stratosphere is determined by a dynamic balance among processes that produce and destroy it and transport it to the troposphere." Thus, the actual role of MC in stratospheric processes can best be elucidated through an examination of multiple perturbation scenarios involving all the known key contributory factors (National Research Council, 1982). The potential of MC, however, can be examined in a context apart from other perturbation processes. In this manner, important information concerning the atmospheric chemistry of MC can be used to develop more representative multiple perturbation simulations. The development of such models has been recommended by the Council to describe the combined effects of all relevant compounds on stratospheric O_3.

A tropospheric lifetime of 5 to 10 years for MC, as estimated by the WMO (World Meteorological Organization, 1982) suggests that a portion of the amount of MC released to the atmosphere reaches the stratosphere. Singh et al. (1982) have estimated that as much as 15 percent of the MC released enters the stratosphere. The National Research Council (1979a), using 1976 global release rates, estimated that about 12 percent reaches the stratosphere. Photodestruction of MC in the stratosphere would increase the atomic chlorine burden, thus accelerating O_3 destruction. Preliminary information reported by Fabian et al. (1981) suggests that MC is rapidly decomposed in the stratosphere. This mixing ratio profile awaits verification.

Reduced O_3 concentrations associated with MC photodestruction could result from the following reaction mechanisms:

$$CH_3CCl_3 \xrightarrow{hv} CH_3CCl_2\cdot + Cl\cdot \qquad (1)$$

$$Cl\cdot + O_3 \longrightarrow ClO + O_2 \qquad (2)$$

$$ClO\cdot + O \longrightarrow Cl\cdot + O_2 \qquad (3)$$

$$ClO\cdot + NO \longrightarrow Cl\cdot + NO_2 \qquad (4)$$

The atomic chlorine produced in reaction (1) would react with O_3 to yield chlorine oxide. The subsequent chain reaction, if not counterbalanced, could result in a continual depletion of O_3.

The modelling results shown in Table 3-4 indicate the efficiency of MC to reduce O_3 relative to other stratospherically important compounds (U.S. Environmental Protection Agency, 1980). It must be noted that such results do not take into account multiple perturbations that occur, some of which could offset the calculated effects of MC.

TABLE 3-4. RELATIVE EFFICIENCY OF HALOCARBONS IN REDUCING STRATOSPHERIC OZONE (U.S. Environmental Protection Agency, 1980)

Compound	Percent ozone depletion after 320 years*
CFC-11	-10.7
CFC-12	-8.5
CFC-113	-8.25
CFC-114	-5.38
MC	-0.93[a]
	-1.6[b]

*production rate assumed, for each species individually, at 1,000 million pounds annually

[a] based on 4.5 year lifetime

[b] based on 9 year lifetime

The World Meteorological Organization (1982), calculated an estimated steady-state depletion of the total O_3 column due to only MC of 0.8 percent. This estimate included an assumption of continued release at the current estimated emissions rate for MC and relied upon the one-dimensional model of the Lawrence Livermore Laboratory (Wuebbles and Chang, 1981) that incorporated revised reaction rate kinetics. It should be noted that other scenarios reported by the WMO, involving other reactive species, did not show a calculated net reduction in total column O_3.

The recent model calculations of Owens et al. (1982) suggest that natural and anthropogenic emissions of methane may significantly moderate the O_3 destroying potential of chlorofluorocarbons. Using a I-D model with chemical reaction rates and incident solar fluxes recommended by WMO (1982), Owens and coworkers have calculated that a doubling of methane, if viewed in isolation, can lead to a total column O_3 increase of 3.5 percent. When coupled with chlorofluorocarbons, the calculation shows an overall total column O_3 change of -1.6 percent. Khalil and Rasmussen (1982) also recently suggested that an increase in the atmospheric levels of methane may serve to protect O_3 levels in the stratosphere. After examining measurement data as far back as 1965, they find the data consistent with a rate of increase in methane ranging from 1.2 to 2 percent per year.

Although a variety of scenarios involving multiple perturbations have been evaluated by the WMO (1982), it is difficult to assess with confidence the actual effect of MC in quantitative terms. To date, measurements of stratospheric O_3 have not detected any depletion. The current levels of calculated O_3 depletion are too small to be observed by existing techniques, which could detect a 2 percent change in total column O_3 if it occurred. Among the confounding factors are the uncertainties and limitations of the models and the complexity of rapidly-changing knowledge in atmospheric chemistry. The NRC (National Research Council, 1982) stated that "These results should be interpreted in light of the uncertainties and insufficiencies of the models and observations." An evaluation of the impact of MC upon stratospheric O_3 must take into account all factors affecting atmospheric processes if realistic estimates of O_3 perturbation are to be made.

3.3.3 Laboratory Studies

In experiments designed to characterize the UV absorption spectrum for MC in the wavelength and pressure ranges associated with the stratosphere, Vanlaethem-Meurée et al. (1979) found that photo-dissociation was the dominant sink process at altitudes above 25 kilometers.

In Spence and Hanst's (1978) photooxidation studies, when MC was irradiated at maximum intensities of 3100 Å and 3650 Å, one-fifth of the initial amount (10 ppm) was consumed after 6 minutes. The principal products observed were carbon monoxide (1.5 ppm), hydrochloric acid (6 ppm), and phosgene (2 ppm).

The recent reaction rate studies, involving MC and OH, by Jeong and Kaufman (1979) and by Kurylo et al. (1979), indicate that the rate of this key reaction is slower and more temperature sensitive than previously had been indicated. Jeong and Kaufman (1979) measured reaction rates using the discharge-flow method and MC which had been extensively purified. A reaction rate at 293°K was determined at $1.06 \pm 0.09 \times 10^{-14}$ cm^3 sec^{-1}. The Arrhenius expression, within 95 percent confidence limits but not including systematic errors, was $5.49 \pm 1.40 \times 10^{-12} \exp [- (1832 \pm 98/T]$ cm^3 sec^{-1}. The authors indicated that the most recent rate (at 298°K) reported by JPL (Jet Propulsion Laboratory, 1979), 1.9×10^{-14} cm^3 sec^{-1}, is too high by a factor of about 1.7 at this temperature, and by a factor of 1.9 at 265°K, the temperature used by Singh (1977) in deriving average OH concentrations.

The Arrhenius expression determined by Kurylo et al., (1979) was in close agreement with that determined by Jeong and Kaufman (1979). Kurylo et al. (1979) used the flash-photolysis method and also extensively purified the MC prior to use.

3.4 MIXING RATIOS IN THE ATMOSPHERE

3.4.1 Global Atmospheric Distributions

Before 1977, reliable estimates of globally averaged MC concentrations were not available. Recent measurements, however, indicate that the global average concentration is consistent with a long (5 to 10 years) tropospheric lifetime. Point measurements of MC mixing ratios in the troposphere are shown in Table 3-5. Typical ambient levels are in the 0.1 to 1 ppb (0.54×10^{-3} to 5.4×10^{-3} mg/m^3) range (Brodzinsky and Singh, 1982). These authors have evaluated the quality of all ambient data reported for MC between 1970 and 1980 and consider it to range from good to excellent. Compared to the levels reported in Table 3-5, dispersion models that have been used to estimate population exposure to ambient MC predict the maximum annual average to which

TABLE 3-5. AMBIENT AIR MIXING RATIOS OF METHYL CHLOROFORM MEASURED AT SITES AROUND THE WORLD

Location	Type of Site	Date	Maximum (ppb)	Minimum (ppb)	Average (ppb)	Reference
Alaska (70°N)		8/79 - 1/81			≃ 0.155	Rasmussen and Khalil, 1981b
Antarctica						
South Pole	Remote	1/75			0.054	Robinson, 1978
South Pole	Remote	1/76			0.057	Ibid.
South Pole	Remote	1/77			0.070	Ibid.
South Pole	Remote	10/77 - 11/77			0.082	Ibid.
South Pole	Remote	1/78			0.083	Ibid.
South Pole	Remote	1/79			0.095	Khalil and Rasmussen, 1980
South Pole	Remote	Jan. 1979 - Jan. 1961	≃ 0.12	≃ 0.094		Rasmussen and Khalil, 1981b
South Pole	Remote	1/80			0.103	Ibid.
Arizona						
Grand Canyon*	Remote	1/28 - 12/5/77	0	0	0	Pellizzari, 1979a
Phoenix	Urban	4/23-5/6, 1979	2.814	0.198	0.824 ± 0.597	Singh et al., 1981, 1982
Arkansas						
El Dorado*		6/13 - 7/8/77	15	2.1	4.7 ± 4.0	Battelle, 1977
Helena	Remote	11/30/76	≤ 1	≤ 1	≤ 1	Ibid.
California						
Badger Pass	High altitude	5/5-13, 1977	0.342	0.130	0.231 ± 0.056	Singh et al., 1978c
Claremont	Urban	9/78	5	0.3	1.2	Cronn et al., 1979
El Cajon*		4/9/75	0.72	-	-	Su and Goldberg, 1976
La Jolla*		4/9/74 - 1/6/76	1.1	0.1	0.34 ± 0.30	Su and Goldberg, 1973
Los Angeles	Urban	4/9-4/21, 1979	5.143	0.224	1.028 ± 0.646	Singh et al., 1981, 1982
Los Angeles	Urban	4/28-5/4, 1976	7.663	0.100	1.545 ± 1.538	Ibid.
Los Angeles Basin	Urban-suburban	4/9-4/21, 1974	2.14	0.01	0.37 ± 0.05 (24-hour)	Simmonds et al., 1974
Lytton Lake*		12/29/75	0.070	-	-	Su and Goldberg, 1976
Mill Valley	Background subject to urban transport	1/11-27/77	0.895	0.107	0.313 ± 0.130	Singh et al., 1979a
Mt. Cuyamaca*		3/15/75	0.41	-	-	Su and Goldberg, 1976
Oakland	Urban	6/28-7/10/79	0.967	0.143	0.291 ± 0.161	Ibid.
Orange City*		4/16/74	0.38	0.38	0.38 ± 0.27	Su and Goldberg, 1976
Palm Springs	Suburban	5/5-11/76	0.545	0.075	0.159 ± 0.075	Ibid.
Point Arena	Marine	5/23-30/77	0.150	0.083	0.111 ± 0.018	Singh et al., 1979a
Point Arena	Marine	8/30-9/5/78	0.158	0.115	0.132 ± 0.011	Ibid.
Point Reyes	Marine	12/1-12/75	0.212	0.061	0.111 ± 0.027	Singh, et al., 1979a
Riverside	Urban	4/25-5/4/77	3.012	0.282	0.834 ± 0.560	Singh et al., 1979a
		7/2-12/80	1.34	0.205	0.747 ± 0.247	Singh et al., 1982

TABLE 3-5. (continued)

Location	Type of Site	Date	Maximum (ppb)	Minimum (ppb)	Average (ppb)	Reference
California						
San Bernadino*	Remote	10/6/76	0.05	-	-	Simmonds et al., 1974
San Jose	Urban	8/21-27/78	2.931	0.150	0.789 ± 0.649	Singh et al., 1979a
San Francisco	Free troposphere	4/77			0.12 ± 0.01	Cronn, et al., 1977a
Santa Monica*	Urban	4/6/74	1.6	-	-	Su and Goldberg, 1976
Stanford Hills	Background subject to urban transport	11/23-30/75	0.565	0.070	0.141 ± 0.117	Ibid.
Upland*		8/13-9/23/77	9.5	0	1.3 ± 3.2	Pellizzari, 1979
Yosemite	High altitude	5/12-18/76	0.126	0.073	0.104 ± 0.009	Ibid.
Colorado						
Denver	Urban	6/15-26/80	2.70	0.171	0.713 ± 0.553	Singh, et al., 1982
Delaware						
Delaware City	Urban	7/8-10/74	0.30	0.03	0.10	Lillian et al., 1975
Hawaii						
Cape Kumakahi	Remote	11/79 - 1/81			≈ 0.14	Rasmussen and Khalil, 1981b
Illinois						
Chicago	Urban	4/21-30/81	0.909	0.241	0.476 ± 0.158	Singh et al., 1982
		4/19/74	0.32	0.2	0.26 ± 0.085	Su and Goldberg, 1976
Ireland						
Western Ireland	Remote	6 & 7/74	---	---	0.048 ± 0.0172	Lovelock, 1974
Japan						
Tokyo	Urban	9/10 - 10/27/75	20.60	0.20	1.70 ± 1.70	Tada et al., 1976
Tokyo		4/74 - 5/74	---	---	0.800	Ohta et al., 1977
Kansas						
Jetmar	Remote	6/1-7/78	0.159	0.106	0.130 ± 0.016	Singh, et al., 1979a
Louisiana*						
Baton Rouge	Urban	1 & 3/77	0.48	0	0.25 ± 0.19	Pellizzari et al., 1979
Lake Charles	Urban	12/6/76 - 6/26/78	6.5	0.25	0.32 ± 0.14	Pellizzari et al., 1979
Plaquemine	Urban		0.32	0.32	0.32 ± 0.14	Ibid.

TABLE 3-5. (continued)

Location	Type of site	Date	Maximum (ppb)	Minimum (ppb)	Average (ppb)	Reference
Maryland						
Baltimore	Urban	7/11-12/74	0.21	0.044	0.12	Singh, et al., 1979a
Missouri						
St. Louis	Urban	5/30 - 6/8/80	0.896	0.132	0.235 ± 0.136	Singh et al., 1982
New Jersey						
Bayonne	Urban	3 - 12/73	14.4	0.075	1.59	Ibid.
Bridgeport	Urban	9/22/77	0.12	0.024	0.072 ± 0.068	Pellizzari and Bunch, 1979
Burlington*	Urban	9/19/77	0	0	0	Pellizzari and Bunch, 1979
Carlstade*	Urban	9/28-30/78	5.6	3.0	4.4 ± 1.3	Pellizzari et al., 1979
Clifton*	Urban	3/22/76				Pellizzari, 1978a
Deepwater*	Urban	6/23-24/77	0.13	0.13	0.13 ± 0.19	Pellizzari, 1978b
Edison*	Urban	3/24/76 - 9/24/78	11	0	4.2 ± 4.2	Pellizzari, 1978a; Pellizzari et al., 1979
New Jersey						
Fords*	Urban	3/26/76 - 9/27/78	6.2	0.24	3.5 ± 2.5	Pellizzari, 1978a; Pellizzari et al., 1979
Hoboken*	Urban	3/23/76	0	0	0	Pellizzari, 1978a
Linden*	Urban	6/21-22/77	0.086	0.086	0.086 ± 0.2	Pellizzari, 1978a
New Brunswick*	Urban	1973	0.27	0.27	0.27 ± 0.025	Lillian and Singh, 1974
Rahway/Woodbridge, Boundbrook, Passaic	Urban	9/20-22/78	20.5	trace	11.4	Pellizzari et al., 1979
Sandy Hook	Marine	7/2-5/74	0.33	0.030	0.15	Lillian et al., 1975.
Seagirt	Marine	6/18-19/74	0.20	0.044	0.10	Ibid.
New York						
New York City	Urban	6/27-28/74	1.6	0.10	0.61	Ibid.
Niagara/Falls/ Buffalo	Urban	7/78	1.0	0.26	0.66	Pellizzari et al., 1979
Staten Island	Urban	3/26 - 4/5/81	1.43	0.22	0.468 ± 0.248	Singh et al., 1982
White Face Mountains	High altitude	9/16-19/74	0.13	0.032	0.067	Lillian et al., 1975
Nevada*						
Reese River	Remote	5/14-20/77	0.14	-	-	Singh et al., 1979a
Ohio						
Wilmington	Urban	7/16-26/74	0.35	0.030	0.097	Lillian et al., 1975

TABLE 3-5. (continued)

Location	Type of Site	Date	Maximum (ppb)	Minimum (ppb)	Average (ppb)	Reference
Oklahoma*						
Liberty Mound	Background	7/10 - 9/21/77	0	0	0	Pellizzari, 1978a
Tulsa	Urban	7/11 - 9/21/77	0	0	0	Ibid.
Vera	Background	9/21/77	0	0	0	Ibid.
Oregon						
Cape Meares	Remote	1/79 - 1/81			≈ 0.155	Rasmussen and Khalil, 1981b
Panama	Remote troposphere	7/77			0.97 ± 0.05	Cronn and Robinson, 1979
Pennsylvania						
Bristol*	Urban	8/19/77	0	0	0	Pellizzari and Bunch, 1979
Marcus Hook*	Urban	8/22/77	0.15	-	-	Ibid.
Philadelphia*	Urban	8/21/77	0.015	-	0.015 ± 0.2	Ibid.
Pittsburgh	Urban	4/7-17/81	1.60	0.158	0.486 ± 0.272	Singh et al., 1982
Samoa (14°S)	Remote	2/80 - 1/81			≈ 0.155	Rasmussen and Khalil, 1981b
Soviet Union						
Caucasus Mountains	Remote	7/79	0.12	0.15	0.13 ± 0.06	Cronn, 1980b
Tasmania						
Cape Grim	Remote	10/76 - 3/77 1/79 - 6/80	---	---	0.012 ± 0.003 ≈ 0.1	Fraser and Pearman, 1978 Rasmussen and Khalil, 1981b
Tennessee						
Smoky Mountains	Regionally polluted	9/78	0.32	0.11	0.10 ± 0.03	Cronn and Harsch
Texas						
Aldine*	Suburban	6/22 - 10/20/77	0.18	0	0.061 ± 0.11	Pellizzari et al., 1979
Deer Park	Urban	7/29-30/76	0.11	0.026	0.089 ± 0.042	Ibid.
El Paso*	Urban	4/5 - 5/1/78	1.0	0.37	0.64 ± 0.26	Ibid.
Freeport*	Urban	8/9 - 11/10/76	2.9	0.44	1.6 ± 0.95	Pellizzari, et al., 1979; Battelle, 1977
Houston	Urban	5/14-24/80	1.50	0.134	0.353 ± 0.263	Singh et al., 1982

TABLE 3-5. (continued)

Location	Type of Site	Date	Maximum (ppb)	Minimum (ppb)	Average (ppb)	Reference
Texas						
Houston,	Urban	7/2/76 - 5/24/80	2.2	0	0.76 ± 0.57	Pellizzari et al., 1979;
Laporte*	Urban	8/12-13/76	1.9		1.9 ± 0.14	Ibid.
Pasadena*	Urban	7/28/76	0	0	0	Ibid.
Utah						
Magna*	Background	10/24 - 11/3/77	0	0	0	Pellizzari, 1979a
Virginia*						
Front Royal	Suburban	9/29 - 11/16/77	0.080	0	0.03 ± 0.037	Pellizzari, 1978a
Washington						
Auburn*	Suburban	1/10 - 11/77	5.2	3.4	4.7 ± 0.81	Battelle, 1977
Pullman	Remote	1/75			0.090	Robinson, 1978
Pullman	Remote	1/76			0.098	Ibid
Pullman	Remote	1/77			0.109	Ibid
Pullman	Remote	10/77 - 11/77			0.115	Ibid
Pullman	Remote	1/78			0.120	Ibid
Pullman	Remote	1/79			0.135	Khalil and Rasmussen, 1980
Pullman	Remote	1/80			0.157	Ibid
Pullman	Remote troposphere	3/76			0.095 ± 0.008	Cronn et al., 1976
Spokane	Urban	4/78			0.5	Cronn et al., 1979
West Virginia*						
Charleston	Urban	9/27 - 11/20/77	51	0	13 ± 25	Pellizzari, 1978a
Institute	Background	11/17-18/77	64	0	32 ± 45	Ibid.
South Charleston	Urban	3/17 - 11/18/77	0.92	0	0.37 ± 0.31	Pellizzari, 1978a, 1979a
St. Albans	Background	9/27 - 10/25/77	0	0	0	Pellizzari, 1978a
West Belle	Background	9/27 - 11/18/77	0	0	0	Ibid.

*Data obtained from summary report of Brodzinsky and Singh, 1982.

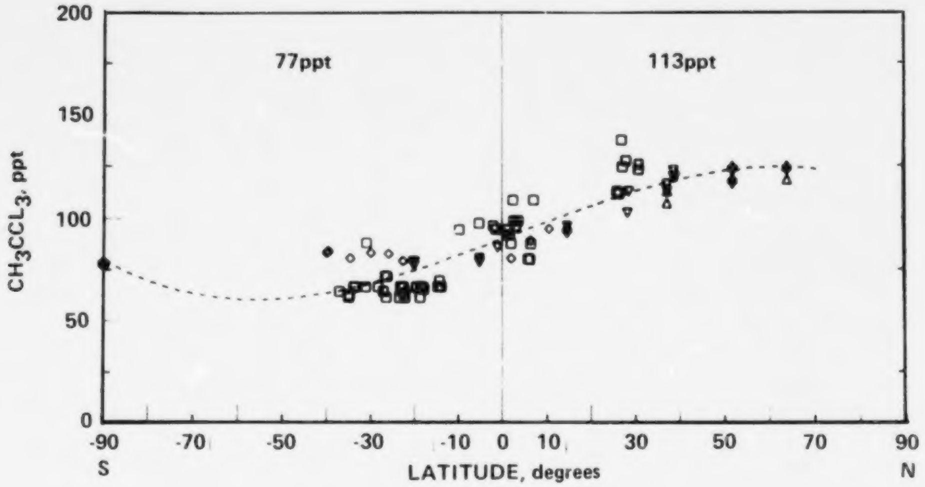


Figure 3-1. Global distribution of methyl chloroform.

Source: Singh et al. (1979a).

people may be exposed is 9.2 ppb (0.050 mg/m^3). Exposure to atmospheric emissions from degreasing operations, the source category of greatest nationwide emissions, is not expected to exceed 4.6 to 9.2 ppb (0.025 to 0.050 mg/m^3) averaged over a year's time. These predicted values are lower than those levels reported (for shorter averaging times) at some urban sites in the United States.

The global average background mixing ratio of MC has been determined by several laboratories, including SRI International and Washington State University. The field studies of Singh and co-workers at SRI have been reported in a number of publications (Singh, 1977a; Singh et al., 1977c; 1978a; 1978b; 1978c; 1979a; 1981; Singh et al., 1982). The global average for late 1977 was reported as 95 ppt (Singh et al., 1979a). This average was derived from measurements made in the northern (average = 113 ppt) and southern (average = 77 ppt) hemispheres. Based on data collected at latitudes 30°N to 40°N from 1975 to 1978, MC presence in the atmosphere was increasing at the rate of 15 ppt (or 17 percent) per year, during this time period.

Field studies by Singh et al. (1979a) were conducted during two oceanographic cruises between latitudes 64°N and 40°S (15 September to 30 October 1977; 20 November to 13 December 1977). Samples were collected in electropolished stainless steel and glass vessels and analyzed by GC-ECD, operated coulometrically. Samples were analyzed in situ as well as after storage. The northern temperate average mixing ratio was reported as 123 ppt. The latitudinal distribution is shown in Figure 3-1.

Rasmussen and Khalil (1981b) reported that tropospheric levels of MC at six locations, in the northern and southern hemispheres between January 1979 and June 1980 were consistent with an estimated lifetime in the range of 6 to 10 years. Analysis was made by EC/GC. From these measurements, a global average concentration of 115 ppt was calculated along with an annual increase of 6.7 ± 2.0 percent. This global average is consistent with the values reported by others for previous years. In contrast, however, to the rate of increase reported by Singh et al. (1979a) for the period 1975 to 1978, the rate of increase appears to be declining, which Rasmussen and Khalil (1981b) attribute to an overall reduction in the rate of emissions. The hemispheric difference in concentration reported by Rasmussen and Khalil (1981b) is consistent with that reported by Singh et al., (1979b). During the period from January 1979 to December 1980, the northern hemisphere average was 130 ppt compared to the southern hemisphere average of 99 ppt (Rasmussen and Khalil, 1981b).

Rasmussen and Khalil (1982) have obtained new estimates of the average mixing ratio of MC in both hemispheres, based on tropospheric air samples collected during the 1978 Project GAMETAG. Based on these samples, the average hemispheric levels are 117±4 ppt (Northern) and 90±3 (Southern). Data for the tropical latitudes indicate that MC levels in boundary air (0.12 to 0.36 km) are 6.4 percent higher than above it. Data limitations precluded a similar analysis for Northern latitudes.

The vertical profile and latitudinal gradients of MC in the troposphere and lower stratosphere have been investigated by Cronn and coworkers in a series of high-altitude studies (Cronn and Robinson, 1979; Cronn et al.; 1976; 1977a; 1977b). Most recently, experiments conducted at the Panama Canal Zone (9°N latitude) and at 37°N indicated that MC tropospheric abundance was 18 percent lower at 9°N (Cronn and Robinson, 1979). Singh et al. (1979a) recorded a 17 percent difference between these latitudes (Figure 3-1). For July

1977, the average tropospheric background concentration (139 whole air samples were collected between ground level and 13.7 kilometers) was 97.3 ± 4.6 ppt at 9°N. At 37°N (Pacific Ocean west of San Francisco), an average mixing ratio of 116 ± 14 ppt was measured. Tropopause height at the Canal Zone was 15.7 kilometers. Methyl chloroform tropospheric mixing ratios did not vary significantly with increasing altitude at 9°N but there was a precipitous drop in the mixing ratio across the tropopause. This observation suggests that modeling of MC's role in stratospheric processes requires a consideration of ultraviolet photoreactivity at all altitudes.

Analysis of pressurized tropospheric samples was performed by a dual GC-ECD. The detection limit for MC was 6 ppt and the precision of analysis was ±4.2 percent. Analyses were performed on 5-ml aliquots. For the low-pressure stratospheric air samples, precision was ±7 percent with a detection limit of 2 ppt. Methyl chloroform was preconcentrated with a freezeout sample loop. Overall accuracy of analysis was estimated at about 10 percent.

In experiments conducted at 37°N (Cronn et al., 1977a), samples were collected at altitudes ranging from 6.1 to 14.3 kilometers with the tropopause height between 11 and 12 kilometers. Samples were analyzed by GC-ECD utilizing the freezeout concentration method. The average tropospheric background mixing ratio for MC in April 1977 (excluding samples within 0.8 kilometer of the tropopause) was 116 ± 14 ppt. This value is consistent with the results of Singh et al., (1979a), who obtained a value of 117 ppt at this latitude during the fourth quarter of 1977 (Figure 3-1).

When this observed mixing ratio (116 ppt) was compared with that obtained in March 1976 at 48°N (Pacific Northwest), an annual atmospheric increase in MC of 23.7 percent was calculated. Cronn et al. (1976; 1977a) collected whole air samples at altitudes ranging from 4.6 to 14.6 kilometers (average tropopause height was 10.8 kilometers) during March 1976 over Western Montana and Idaho. The average tropospheric background mixing ratio for MC was 94.5 ± 8.2 ppt. Samples were analyzed by GC-ECD both isothermally with a 5-ml aliquot and with the freezeout concentration method. Precision of the overall method was ±4 percent.

The Washington State University (WSU) group has made several studies by aircraft of the latitudinal distribution of MC (Cronn, 1980a; Robinson, 1978; Robinson and Harsch, 1978). Interhemispheric differences of MC in the troposphere in June, 1976, were reported, with northern levels of 96 ppt and southern levels of 88 ppt (Robinson and Harsch, 1978). Lower stratospheric levels were 80 and 67 ppt, respectively. Work begun at WSU in 1976, and continuing at both WSU and the Oregon Graduate Center, has provided annual measurements of interhemispheric differences of MC of 1.67, 1.72, 1.56, 1.40, and 1.45 for 1975, 1976, 1977, 1978, and 1979, respectively (Khalil and Rasmussen, 1980; Robinson, 1978). Time-trend monitoring has been conducted nearly continuously at a ground station in eastern Washington state since July 1977 (Cronn, 1980a). Increases have exceeded 12 percent per year during that time.

Point measurements made by Lovelock (1977b) during 1972 to 1977 resulted in a lower global average background mixing ratio (73 ppt). Air samples collected at rural sites in the British Isles indicated that, during the 5 year period, MC mixing ratios increased from 31 ppt to 97 ppt. In the southern hemisphere (Africa and Antarctica), it increased from 12 ppt to 50 ppt. Absolute accuracy of the GC-ECD method was reported as ±30 percent.

In field measurements of ambient MC levels over North America (18°N to 65°N) from October 4 to 13, 1976, Pierotti and co-workers (1980) determined an average tropospheric mixing ratio of 145 ± 25 ppt for this region. The mixing ratio dropped sharply across the tropopause and in the stratosphere, suggesting a large sink at these altitudes. These investigators suggested that the data were consistent with a northern hemisphere background mixing ratio of about 100 ppt.

3.5 LEVELS FOUND IN WATER

Groundwater data available from state sources indicate that 23 percent of the 1,611 wells tested contained MC (Coniglio, 1981). Concentrations of 344 ppb were measured in discharge waters from a MC manufacturing facility (Battelle, 1977). Chow (1981) reported a level of 700 ppb in testing of residential wells near a manufacturing site. The highest level reported for drinking water is 17 ppb (Battelle, 1977). Chian and Ewing (1977) have examined surface water at various sites in the United States and found that 11 of 204 sites had MC levels greater than 6 ppb. The maximum level of MC found was 8 ppb. Bellar et al. (1974) reported that the influent level of MC to a municipal sewage treatment plant was 16.5 ppb; upon treatment, the level dropped to 9.0 ppb. Contamination of community drinking water supplies by MC and other halogenated solvents used as components of cesspool cleaners was reported (U.S.E.P.A., 1979b). It was cited that levels of MC in observation wells were as high as 5,000 ppb. The maximum level of MC found in the contaminated drinking water wells was 310 ppb. Given the high volatility of MC and the generally low levels expected in natural aquatic environments, MC is expected to be nonpersistent.

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4. METABOLIC FATE AND DISPOSITION

4.1 ABSORPTION, DISTRIBUTION, AND ELIMINATION

Methyl chloroform (MC; 1,1,1-trichloroethane) is currently one of the most widely used of the chlorinated aliphatic hydrocarbon solvents. Since its introduction in the mid-1950's as a cold cleaning solvent substitute for carbon tetrachloride, MC has gained recognition as being among the least toxic of the chlorinated aliphatic hydrocarbons (Chemical Marketing Reporter, 1982) and is increasingly replacing other supposedly more toxic chlorinated solvents such as trichloroethylene (Torkelson et al., 1958; Prendergast et al., 1967; Stewart, 1968). The fact that its isomer, 1,1,2-trichloroethane, is markedly more toxic than MC (Hardie, 1964; Irish, 1963; Browning, 1965; Carlson, 1973), is in agreement with the general observation that chlorinated ethanes having chlorines on both carbon atoms are considerably more soluble in water, blood, and lipid (Table 4-1). These physical properties could be responsible in part for the manifestations of differences in chlorinated hydrocarbon toxicity (Sato and Nakajima, 1979; Clark and Tinston, 1973).

TABLE 4-1. PARTITION COEFFICIENTS OF METHYL CHLOROFORM AND OTHER SOLVENTS AT 37°C

Compound	Vapor Press torr at 25C	Water/Air	Olive Oil/Air	Blood/Air	Olive Oil/Blood
1,1,1-Trichloroethane	125	0.93	356	3.3	108
1,1,2-Trichloroethane	25	17.1	2273	38.6	59
1,1-Dichloroethane	250	2.7	187	4.7	40
1,2-Dichloroethane	80	11.3	447	19.5	23
Trichloroethylene	436	1.3	718	9.5	76
Tetrachloroethylene	20	0.43	1917	13.1	146
Dichloromethane	400	7.6	152	9.7	16
Chloroform	250	3.5	401	10.3	39
Carbon tetrachloride	100	0.25	361	2.4	150

Adapted from Sato and Nakajima, 1979.

4.1.1 Oral and Dermal Absorption

While limited absorption of MC vapor through the lungs is the common route of entry into the body, MC is also rapidly and completely absorbed from the gastrointestinal tract (Stewart, 1971). Stewart and Andrews (1966) reported an instance of non-fatal acute intoxication after oral ingestion of a liquid ounce of MC (0.6 g/kg body weight,BW). The concentration of MC in the expired air was measured serially and found equivalent to an inhalation exposure of experimental subjects to 500 ppm (2,700 mg/m^3). On the other hand, MC vapor is poorly absorbed through intact skin and, unless it is trapped against the skin beneath an impermeable barrier, it is unlikely that toxic quantities can be absorbed (Stewart, 1971; Stewart and Dodd, 1964). Stewart and Dodd (1964) demonstrated, with continuous immersion for 30 min of the thumbs or hands of volunteers, that the solvent penetrates the skin, enters the blood circulation, and is excreted through the lungs in exhaled air. Fukabori and coworkers (1976, 1977) attempted a quantitation of cutaneous absorption in humans. Systemic absorption was evaluated by measurement of MC in blood and exhaled air and its biometabolites [trichloroethanol (TCE) and trichloroacetic acid (TCA)] in urine. After applications of the solvent to the skin of the forearm in a circumscribed area (12.5 cm^2) for 2 hours a day for 5 consecutive days, or immersion of the hands 11 times a day for 10 minute periods, the MC concentrations in exhaled air and urinary metabolites corresponded roughly to a 2-hour inhalation exposure to 10-20 ppm (54 to 108 mg/m^3) MC in ambient air. The investigators concluded that absorption through the skin for workers in direct contact with liquid MC may add to the absorption from vapor exposure.

Additional evidence that MC absorption via skin can be a source of exposure is provided by the results of Jakobson et al. (1982). Application of a stabilized formulation of MC to the skin (3.1 cm) of anaesthetized guinea pigs resulted in peak blood levels (1.9 ug/ml) 30 minutes after exposure. Blood levels then declined to 0.66 ug/ml 6 hours after exposure MC was applied at two sites on shaved skin under an occlusive barrier. With elimination studies, using percutaneous exposure 4 hours in duration, elimination from the blood was consistent with a two-compartment model exhibiting nonlinear kinetics. During exposure, no visible skin reactions were noticed at the application sites. In comparison, Astrand (1975) obtained a level of 4 ug/ml arterial blood after a 30-minute exposure of humans to 245 ppm (1,323 mg/m^3).

4.1.2 Pulmonary Uptake and Body Burden

Several studies have been made of the pharmacokinetics of MC pulmonary uptake, distribution in the body, and metabolism after exposure to low inhalation concentrations approximating the accepted TWA concentration. Information available from these studies concerns single exposures under experimentally controlled conditions with animals or human volunteers. Little information is available regarding long-term exposure, such as may occur in the workplace or in the environment. Recent studies have greater reliability than earlier studies because of the availability of gas chromatographic methods for the analytic determination of MC and its metabolites in alveolar air, body fluids, and tissues (Schumann et al., 1982a,b; Monster et al., 1979b; Monster and Boersma, 1975; Astrand et al., 1973; Eben and Kimmerle, 1974; Briemer et al., 1974; Humbert and Fernandez, 1976, 1977; Stewart et al., 1969), although spectrophotometric methods based on adaptations of the Fujiwara reaction are still widely used by many investigators for determining urinary excretions of trichloroethanol (TCE), trichloroacetic acid (TCA), or total urine chloroderivatives of MC (Ogata et al., 1974; Imamura and Ikeda, 1973; Tanaka and Ikeda, 1968; Ikeda and Ohtsuji, 1972).

Inhaled MC rapidly equilibrates with arterial capillary blood across the lung alveolar endothelium (Astrand, et al., 1973). The rate of pulmonary uptake or absorption depends largely on the solubility of MC in blood (Ostwald solubility coefficient) and hence the blood/air partition coefficient (Table 4-1). Vapors with a high partition coefficient are absorbed into the body readily and may exhibit selective partitioning into various tissues. Conversely, vapors with a low partition coefficient are expected to approach steady-state tissue levels slowly. This is likely since partition occurs more rapidly with venous blood and alveolar air, and thus vapors are more rapidly eliminated into expired air following exposure. In comparison with other common solvents in Table 4-1, MC possesses a relatively low blood/air partition coefficient of about 3.3 at 37°C.

The magnitude of MC uptake (dose, burden) into the body is related to the following factors: 1) concentration of MC in the inspired air; 2) duration of exposure until steady-state is reached; 3) pulmonary ventilation during exposure; 4) blood/ air partition coefficient; 5) rates of diffusion into, and solubility in, the body tissues; 6) total body-lipid repository, and 7) metabolic rate. Consequently, during exposure at a given inspired air concentration, pulmonary uptake and retention is initially large and gradually decreases to a minimum steady-state value as total body equilibrium (and body burden) with inspired air concentration is reached. Under steady-state conditions, pulmonary uptake balances the pulmonary and other routes of elimination, including metabolism. For any given breath cycle during exposure, the proportion of MC absorbed and retained by the body is equal to the inspired air concentration (C_I) minus the end alveolar air concentration (C_A) and, since pulmonary uptake is a function of inspired air concentration, the percent retention is:

$$\text{\% retention of inspired air concentration} = \frac{C_I - C_A}{C_I} \times 100 \qquad (1)$$

This value is large at the beginning of exposure, but gradually decreases as total body equilibrium is approached (Figure 4-1). The percent retention value is independent of the inspired air concentration. During experimental exposures of subjects to 70 and 140 ppm (378 and 756 mg/m^3) MC for 4 and 8 hours, both Monster et al. (1979b) and Humbert and Fernandez (1977) found retention to be 30 percent of inspired air concentration at an equilibrium reached after 4 hours of exposure. In a report submitted for publication, Nolan et al. (1983) reported that 25% of the MC inhaled by six volunteers at either 35 or 350 ppm (139 or 1,890 mg/m^3) during a 6-hour exposure, was retained.

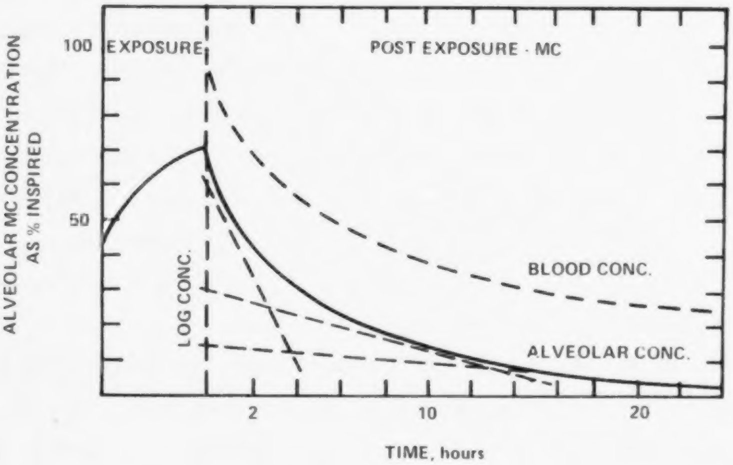


Figure 4-1. Absorption and pulmonary elimination of MC, and blood concentration (see text for explanation).

Source: Davidson (1980).

The total amount (dose, Q) of MC retained in the body during an inhalation exposure can be estimated by multiplying percent retention by the volume of air inspired during the exposure period, or:

$$Q = (C_I - C_A)\ \dot{V} \cdot T \qquad (2)$$

where $\dot{V}$ is ventilation rate (ℓ/min) and T is exposure period (min). Since the retention value decreases exponentially during the exposure until equilibrium is reached (Figure 4-1), either experimental measurements of retention at frequent intervals during exposure (Monster et al., 1979b) or integration over the experimentally determined retention function is required (Humbert and Fernandez, 1977). The exponential nature of the retention curve is due to first-order kinetics of saturation of body compartments with MC.

Table 4-2 shows the amounts of MC absorbed into the body during inhalation exposures for volunteers from the studies of Monster et al. (1979b) and Humbert and Fernandez (1977). Monster and his coworkers estimated pulmonary uptake of MC by multiplying the minute volume by percent retention during single 4-hr exposures to 70 ppm and 145 ppm (378 and 783 mg/m^3). They observed a direct proportionality between uptake and inspired air concentrations of MC. Similar results were obtained by Humbert and Fernandez for volunteers exposed for 8 hours to 72 ppm and 213 ppm (389 and 1,150 mg/m^3). Comparison of pulmonary uptake for 4-hr and 8-hr (Table 4-2) exposures indicates that the amount of MC retained is also proportional to duration of exposure until a steady-state is reached. Although the values for uptake determined by Humbert and Fernandez are 40 percent lower than those of Monster et al, this variation can be ascribed to differences in the experimental methodologies and average minute volume. Nonetheless, these experimental results indicate that the body burden resulting from an 8-hr inhalation exposure to 350 ppm (1,890 mg/m^3) (TWA) approximates 1.5 to 2 g of MC for a normal 70-kg man.

The body uptake of MC increases with the duration of inhalation exposure and with physical work or exercise. Monster et al. (1979b) found that during a 4-hr exposure to 142 ppm (767 mg/m^3) MC, with physical activity equivalent to light physical work (100 watts), pulmonary uptake increased from 429 mg to 538 mg MC, an increase of 25 percent (Table 4-2). This increase was primarily due to an increase of ventilation from 10.7 ℓ$\min^{-1}$ sedentary to 30.6 ℓ$\min^{-1}$ with work. While it might be expected from equation (2) that the increased

TABLE 4-2. ESTIMATED UPTAKE OF MC DURING A SINGLE 4-HR INHALATION EXPOSURE (AVERAGE BODY WEIGHT, 77 kg; 67 kg LEAN BODY MASS), MONSTER ET AL., 1979b

Subject	Exposure Concentration		
	70 ppm (at rest*) Uptake, mg	145 ppm	142 ppm* with work (100 watt) Uptake, mg
A	140	305	435
B	240	520	610
C	200	465	540
D	185	395	560
E	200	425	575
F	190	465	505
Av.	193	429	538

*Ventilation minute volume increased from average of 10.7 ℓ/min. at rest to 30.6 ℓ/min during light work.

ESTIMATED UPTAKE OF MC DURING A SINGLE 8-HR INHALATION EXPOSURE (AVERAGE BODY WEIGHT 74 kg), HUMBERT AND FERNANDEZ, 1977

Subject	Exposure Concentration*	
	72 ppm Uptake, mg	213 ppm
BH	293	921
JC	274	912
JO	277	--
Av.	281	917

*Average ventilation minute volume 5.7 ℓ/min.

uptake should be directly proportional to the ventilation rate increment, an increase in ventilation also tends to increase alveolar elimination of MC from pulmonary venous blood. Similar observations were made by Astrand et al. (1973) with volunteers exposed to an MC inhalation concentration of 250 and 350 ppm (1,350 and 1,890 mg/m^3) for 30 minutes at rest, alternating with identical exposure plus 50 watts of light work. Table 4-3 shows that their subjects responded to this work with a 3-fold increase of pulmonary ventilation, nearly a 2-fold increase in cardiac output, and a 50 percent increase in arterial blood concentration, which is an index of pulmonary uptake. Also, end alveolar concentration of MC increased while retention decreased from 50

TABLE 4-3. MEAN VALUES AND SEM FOR 12 MALE SUBJECTS AT REST AND EXERCISE FOR 30 MINUTE PERIODS

MC	Ventilation BTPS ℓ/min	Cardiac output ℓ/min	Alveolar conc ppm	Blood arterial conc. μg/g
At rest				
250 ppm	6.6 ± 0.4	5.1 ± 0.4	125 ± 6	3.0 ± 0.2
350 ppm	6.6 ± 0.4		179 ± 13	5.0 ± 0.5
50 Watt				
250 ppm	22.5 ± 1.0	9.7 ± 0.6	168 ± 7	4.5 ± 0.2
350 ppm	21.8 ± 1.1		239 ± 17	7.2 ± 0.4

From Astrand et al. (1973).

to 33 percent with work. Therefore, physical activity during MC exposure increases the uptake, but the increase is not directly proportional to increased ventilation and is self-limited by a compensatory increase of pulmonary elimination. For solvents like MC, with a low blood/air partition coefficient, physical activity has a smaller effect on net pulmonary uptake and body retention than those solvents with a higher blood/air partition coefficient (e.g., trichloroethylene, perchloroethylene, dichloromethane; Table 4-1; Monster, 1979).

During inhalation of MC, and in the elimination phase after exposure, the concentration in arterial blood leaving the lung always is directly proportional to the alveolar concentration (Monster et al., 1979b; Humbert and Fernandez, 1977; Astrand et al., 1973; Gamberale and Hultengren, 1973; Eben and Kimmerle, 1974). Since at equilibrium the end alveolar air concentration is proportional to inspired air concentration, blood concentration of MC is also related to inspired air concentration (Monster et al., 1979b; Humbert and Fernandez, 1977; Astrand et al., 1973). This fixed relationship between alveolar air and blood concentration is defined by the blood/air partition coefficient for MC. Figure 4-2 illustrates this linear relationship for a volunteer exposed for 30 minutes to increasing increments of MC concentration

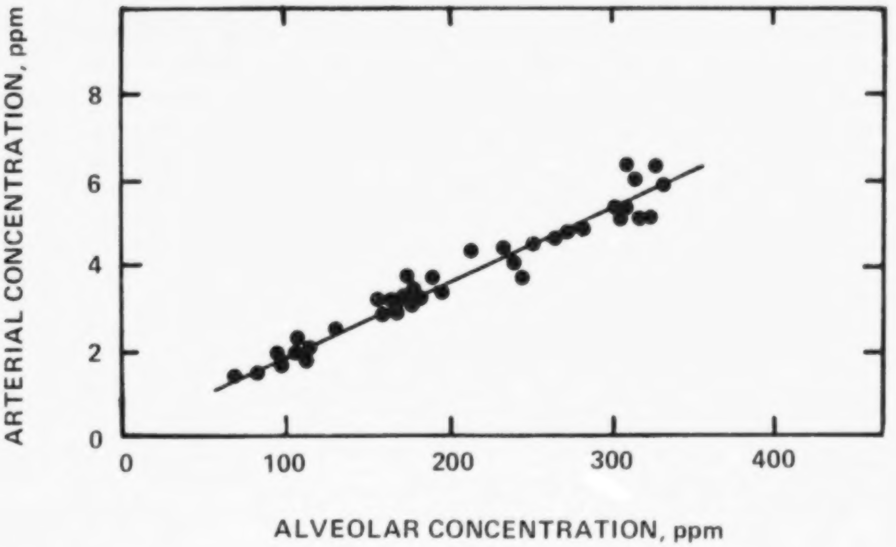


Figure 4-2. Relationship between methyl chloroform concentration in alveolar air and arterial blood. Data from one subject. Product moment correlation: r = 0.985. Data from one subject exposed to 30-min. periods of MC. Alveolar air samples and arterial blood samples.

Source: Gamberale and Hultengren (1973).

in inhaled air. The relationship was independent of the duration of exposure over the time period studied. Eben and Kimmerle (1974) exposed rats to 204 ppm (1,101 mg/m^3) for 8 hours daily, 5 days a week for 14 weeks and found that the blood concentration of MC determined immediately after daily exposure remained constant during the entire 3 month period, suggesting that steady-state was attained.

The blood/air partition coefficient, as determined from in vivo measurements of alveolar air concentration and blood concentration of MC, agrees well with the in vitro value of 3.3 at 37°c determined by Sato and Nakajima (1979) (Table 4-1). Table 4-3 summarizes the data of Astrand et al. (1973), determined for sedentary men exposed to 250 and 350 ppm (1,350 and 1,890 mg/m^3) MC for 30-min periods alternating with 30-min periods of physical activity. The ratios of arterial blood concentration (μg/g) to alveolar MC concentration remain nearly constant over a 2-fold range of alveolar concentrations, with an average value of 5. Monster et al. (1979b) estimated from their study of men exposed to 70 and 142 ppm (378 and 767 mg/m^3) MC a blood/alveolar air concentration ratio of 6. In comparison to other solvents (Table 4-1), MC has a relatively small blood/ air partition coefficient and, hence, for equivalent ambient air exposure concentrations the blood concentration of MC is proportionally lower than for other solvents.

The amount of MC pulmonary uptake is influenced by total body weight and also by the total fat content of the body (average body fat = 8 percent of body weight). The capacity of adipose tissue to absorb MC _in vivo_ is determined by the product of adipose tissue volume and the lipid solubility of MC. The olive oil/blood partition coefficient for MC (108 at 37°C) is higher than for most other structurally related solvents (Table 4-1) and, therefore, the capacity of adipose tissue for MC is relatively high. However, because of the low rate of perfusion (5 percent of cardiac output), the time needed to saturate adipose tissue is large in comparison with that for other tissues. Also, since the blood/air partition coefficient for MC is lower than that of other solvents, the amount of MC in adipose tissue (concentration) at the end of an exposure of similar duration will be relatively lower. Monster (1979) suggests that because of the high solubility of MC in adipose tissue, tissue storage of MC under non-steady state conditions will vary with repeated daily exposures, particularly in obese persons. This concept is supported by the observations of Savolainen et al. (1977) and Vainio and coworkers (1978), who exposed rats to MC (500 ppm; 2,700 mg/m^3) 6 hr daily for 5 days and determined MC concentrations in perirenal adipose tissue and in other body tissues. Their data, shown in Table 4-4, indicate that measureable amounts of MC remained in perirenal fat tissue 18 hours after the previous exposure of day 4, and markedly increased further with a 6-hr exposure on day 5. The adipose tissue/blood partition coefficient calculated from these data was 21, as compared to 1.6 for brain and liver tissues. On the other hand, Eben and Kimmerle (1974) exposed rats for 14 weeks (8 hr daily, 5 day per wk) to 204 ppm (1,101 mg/m^3) MC but failed to find MC in adipose or other tissues and concluded that MC did not accumulate with chronic exposure. It should be noted that low-level, prolonged exposures, such as encountered in the ambient environment, would be expected to result in steady-state tissue concentrations. Under these conditions no further MC would be expected to partition into lipid-rich tissues.

TABLE 4-4. TISSUE CONTENT (RAT) OF METHYL CHLOROFORM (MC) AFTER CHRONIC INHALATION EXPOSURE OF 500 ppm*

Exposure on 5th day hr.	Cerebrum	Cerebellum	Liver	Perirenal fat	Blood
	nmolMC/g wet weight ± range				
0	0.15 ± 0.03	0.17 ± 0.03	0.15 + 0.01	16.9 + 0.5	0.08 ± 0.01
2	14.6 ± 1.1	14.0 ± 2.2	14.7 ± 0.04	183.5 ± 10.7	11.5 ± 2.0
3	13.4 ± 0.60	13.2 ± 0.8	15.7 ± 3.3	218.9 ± 63.4	8.5 ± 1.0
4	12.2 ± 0.40	15.9 ± 1.5	16.2 ± 2.8	261.2 ± 19.1	12.7 ± 2.9
6	15.6 ± 4.6	21.3 ± 9.6	21.3 ± 0.4	276.0 ± 30.1	13.1 ± 1.9

*Measurements were performed on the 5th day of exposure after 4 previous daily exposures, 6 hr daily.

From Savolainen et al. (1977).

4.1.3 Tissue Distribution

During exposure to MC, distribution of the compound throughout the body and the amount concentrated by each tissue is primarily governed by the blood concentration, the blood perfusion rate, and the affinity of MC for respective tissues, as determined by individual tissue/ blood partition coefficients. In comparison with other chlorinated hydrocarbon solvents that are known to be distributed widely in the body, MC has one of the highest lipid/ blood partition coefficients (108 at 37°C, Table 4-1) and would distribute into all body tissues, particularly those high in lipid content, such as brain and adipose tissue (Holmberg et al., 1977). Table 4-4 shows the concentrations of MC in liver, brain, and adipose tissue of rats exposed to 500 ppm (2,700 mg/m^3) in their inspired air for 6 hours daily for 5 days (Savolainen et al., 1977). Adipose tissue appears to have a partition coefficient with blood of approximately 20, and brain tissue levels are also greater than blood concentration. In man, MC readily passes the blood-brain barrier, as evidenced by the concentrations in the brain reported by Caplan et al., (1976) and in the cerebrospinal fluid (Larsby et al., 1978). While it has not been demonstrated directly to cross the placental barrier into the fetus, it may be expected to do so (Laseter and Dowty, 1977) like other highly lipid-soluble haloalkanes. Trichloroethanol (TCE), a major metabolite of MC, is known to cross readily into the fetus (Bernstine et al., 1954, 1957). Leighty and Fentiman, Jr. (1981) have recently provided *in vitro* evidence that TCE, upon incubation in a rat liver microsomal system fortified with coenzyme A, appears to conjugate with palmitic acid. While there is no report of MC occurring in colostrum or milk of nursing mothers, it may possibly distribute into these compartments because of their high lipid content.

4.1.4 Pulmonary Elimination

Figure 4-1 shows schematically the time-course of pulmonary elimination of MC after exposure. At termination of exposure, MC immediately begins to be eliminated from the body into the lungs with blood concentration and alveolar air concentration describing parallel exponential decay curves with three major components. These components represent first-order passive diffusions of MC from three major body compartments: (1) most rapidly from a vessel-rich group of tissues (VRG) with high blood flow and high diffusion rate constant (VRG: brain, heart, kidneys, liver, endocrine, and digestive system), (2) more slowly from the lean body mass (MG; muscle and skin) and (3) from adipose tissue (FG) (Fiserova-Bergerova and Holaday, 1979). The rate constants for the passive diffusion from VRG, MG and FG compartments are dependent on both the arterial blood flow/tissue mass and the relative solubilities of MC in the tissues of these compartments (tissue/ blood partition coefficients). However, the ranking of half-times ($t_{1/2}$) of elimination of MC is VRG < MG < <FG and is independent of the body burden. The areas under the alveolar or blood elimination curves in Figure 4-1 are proportional to the quantity of MC absorbed during exposure. The body burden can be obtained by integrating the exponential functions from the time immediately at the end of exposure to infinity.

The precise half-times of elimination of MC from the three major body compartments have not been well established. Monster et al. (1979b), who exposed subjects to 70 ppm and 145 ppm (378 and 783 mg/m^3) for 4 hours, estimated from decay curves of MC in blood and exhaled air half-times of elimination of 9.2 and 26 hours for the MG and FG compartments, respectively. Humbert and Fernandez (1977) determined the exponential decay of alveolar air concentration after 8 hours exposure of subjects to 72 and 213 ppm (389 and 1,150 mg/m^3) MC. These investigators calculated half-times of elimination for compartments VRG, MG, and FG as 0.8 hours, 7 hours, and 35 hours, respectively. From the postexposure alveolar concentration curves published by Stewart and his associates (Stewart and Andrews, 1966; Stewart et al., 1969; Stewart, 1971) for subjects singly and repeatedly exposed to various inhalation concentrations of MC, the half-time of elimination for MG and FG can be estimated as approximately 14 and 24 hours, respectively. The long half-time of elimination (24 to 35 hours) from the adipose tissue compartment observed by these investigators reflects the high lipid/blood partition coefficient of MC and indicates that traces of MC may be present up to 4 to 5 days. Experimentally, both Monster et al. (1979b) and Humbert and Fernandez (1977) observed that, after single controlled inhalation exposures, pulmonary elimination was not complete for 6 to 10 days as determined by the presence of MC in alveolar air. The possibility of increased partitioning into tissues upon repeated daily exposures to this solvent (Laseter and Dowty, 1977), however, is offset in part by the low blood/air partition coefficient (Table 4-1) which, for a given exposure concentration, limits pulmonary absorption and body dose.

Nolan et al. (1983) reported also that pulmonary elimination of MC in sedentary human volunteers exposed to 35 and 350 ppm (189 and 1890 mg/m^3) was tri-exponential, with half-times estimated at 44 minutes, 5.7 hours, and 53 hours. Over 91% of the absorbed MC was excreted unchanged via the lungs.

4.1.5 Elimination by Other Routes

There is no report in the literature of significant elimination of MC by any route other than pulmonary. MC is poorly soluble in water, even when compared with other chlorinated hydrocarbons (Table 4-1), and with its high lipid/water partition coefficient, MC is unlikely to be excreted unchanged in the urine in any significant amounts. Studies of chlorinated compounds in the urine after exposure of animals and humans to MC have not demonstrated its appearance in urine (Schumann et al., 1982a; Monster et al., 1979b; Eben and Kimmerle, 1974; Humbert and Fernandez, 1977; Seki et al., 1975; Stewart et al., 1961; Hake et al., 1960). Since highly lipid soluble substances like MC readily cross into the intestinal lumen, some fecal and flatus excretion can be expected from inhalation exposures. After controlled inhalation exposures, Humbert and Fernandez (1977) were able to account for 88 to 100 percent of an estimated retained dose as unchanged MC in postexposure exhaled air and as metabolites of MC in urine. However, Monster et al. (1979b) could account for only 60 to 80 percent; furthermore, they noted that the percentage recovered decreased with higher exposure doses.

4.2 BIOTRANSFORMATION

MC has long been known to be metabolized to only a very limited extent by mammals. The generally accepted metabolites of MC -- trichloroethanol (TCE), TCE-glucuronide, and trichloroacetic acid (TCA) -- are excreted primarily by the kidney, but very small amounts of TCE (<1 percent) are excreted by the lungs (Monster et al., 1979b). TCE-glucuronide is also excreted to an unknown extent in bile (Owens and Marshall, 1955a,b).

Filser et al. (1982) evaluated a variety of compounds, including MC, for their ability to stimulate the endogenous production of acetone. This study was based on previous observations that certain haloethylenes caused acetonemia in rats (Filser and Bolt, 1980). Exposure of rats (Filser et al., 1982) to 1,000 ppm (5,400 mg/m^3) MC for up to 50 hours did not stimulate acetone production as measured by gas chromatographic analysis of exhaled air. Furthermore, these investigators reported that the metabolic rate of MC was below their limit of detection. Data supporting this latter observation were not provided.

4.2.1 Magnitude of MC Metabolism

More than 20 years ago, Hake and his coworkers (1960), using ^{14}C-labeled MC, determined that less than 3 percent of MC is metabolized by rats. More recently, estimates of the extent of metabolism in man have been made from controlled inhalation exposures with unlabeled MC (Seki et al., 1975; Monster et al., 1979b; Humbert and Fernandez, 1977; Nolan et al., 1983). From the experimentally determined retained dose and the amounts of MC metabolites excreted into the urine, the percent of the dose metabolized in man is estimated to be no more than 6 percent.

Hake et al. (1960) found that 98.7 percent of a dose of MC given to rats was eliminated unchanged via the lungs. These investigators injected 1,1,1-trichloroethane-1-^{14}C (700 mg/kg) intraperitoneally into 3 rats (170-183 g), which were then placed individually in a Roth metabolism unit with air traps for ^{14}C-MC (cold toluene), $^{14}CO_2$ (NaOH solution) and finally a trap for "metabolites" (quartz-tube furnace and halogen absorber that converted any halogenated hydrocarbon to inorganic halogen and CO_2, and absorbed the halogen). Each rat was kept in the unit for 25 hours, during which time urine and feces were collected. At necropsy at 25 hours, blood and tissue samples were analyzed for total ^{14}C-activity. The ^{14}C-MC used in the experiments was synthesized by the investigators and was determined by paper chromatography to be 99 percent pure, with possible contamination by 0.4 percent 1,1,2-trichloroethane and 0.5 percent 1,1-dichloroethane. Their findings, summarized in Table 4-5, indicate that metabolism of MC in the rat is limited to about 2 percent of a very large dose. However, only TCE-glucuronide was identified as a metabolite in the urine, and the source of the $^{14}CO_2$ in expired air could not be defined because of the presence of small amounts of ^{14}C-1,1,2-isomer and ^{14}C-dichloroethane in the dose.

Recently, Schumann and coworkers (1982a) evaluated pharmacokinetic parameters of ^{14}C-labelled MC to characterize the disposition of the inhaled compound in male Fischer 344 rats and B6C3F1 mice. The animals, ranging in age from 2.5 to 3.5 months, were exposed to 150±9 or 1,500±90 ppm [^{14}C] MC (810 or 8,100 mg/m^3) for 6 hours and elimination of ^{14}C activity was followed for 72 hours. As other investigators have found, Schumann and coworkers also observed that body burden, end-exposure blood levels, and tissue concentrations of MC were found to increase in direct proportion with exposure concentration. It was also observed that, as others have found, that MC was more concentrated in the lipid stores of both species than in the liver or kidneys immediately following exposure. However, only 2 percent or less of the initial radioactivity remained 24 hours after exposure ceased. Thus, it is reasonable to conclude that MC has little potential for bioaccumulation in these species.

TABLE 4-5. HAKE ET AL. (1960) RECOVERY EXPERIMENT WITH RATS (3) INTRAPERITONEALLY INJECTED WITH ^{14}C-MC (700 mg/kg)

	Average % dose
Expired Air	
Unchanged MC (by isotopic dilution)	97.6
Unknown compound detected by furnace (assumed to be unchanged MC)	1.1
$^{14}CO_2$ (in NaOH) 70% within 4 hr; 100% within 12 hr	0.5
Urine	
TCE-glucuronide + other volatile compounds but no detectable TCA	0.85
Feces	
Uncharacterized	0.03
Tissues	
Uncharacterized except skin (90% unchanged MC)	0.18

Schumann et al. observed that between 94 and 98 percent of the total recovered radioactivity in rats and between 87 and 97 percent of that in mice was unchanged MC in expired air. These data are in general agreement with the observations of others reported in this section. The remaining radioactivty consisted of either $^{14}CO_2$ or nonvolatile radioactivity in urine, feces, and carcass.

Of particular interest is the observation by Schumann and coworkers that biotransformation of MC appears to be a saturable and dose-dependent process in both species. Over the 10-fold exposure range, a 2 to 3-fold increase in the mean micromole-equivalent of MC metabolized per kilogram of body weight was observed in both species. Species differences noted were (1) a greater rate of pulmonary clearance of MC in mice relative to rats, (2) end-exposure blood levels, (3) and log-linear elimination constants for two-compartment (rats) and three-compartment (mice) linear models. As the authors concluded, these differences can reasonably be ascribed to species differences in respiratory minute volume and metabolism. Coupled with the observations of Eben and Kimmerle (1974) the results of Schumann and coworkers suggest that saturation of metabolic pathways occurs somewhere in the range of 500 to 1,500 ppm (2,700 and 8,100 mg/m^3) in these rodent species.

The pharmacokinetic description and biochemical basis of saturable metabolism in relation to a variety of organic compounds is comprehensively discussed in a series of reports by Andersen and coworkers (1980) and Andersen (1981a,b,c).

In a companion study, Schumann and coworkers (1982b) determined that the fate of inhaled MC is not altered upon repeated exposure of male Fischer 344 rats and B6C3F1 mice. Both rats and mice were exposed to 1,500 ppm (8,100 mg/m^3) unlabeled MC for 16 months and, on the last exposure, to ^{14}C-MC. The fate of ^{14}C-MC was followed for 72 hours and compared to age-matched controls exposed concurrently to chamber air for 16 months prior to receiving the single ^{14}C-MC exposure. Results showed that prior repeated exposure did not significantly alter the quantity of MC excreted via the pulmonary route compared to those singly exposed, of either species. Although the mean end-exposure body burden of ^{14}C-activity was about 2-fold greater in mice than rats, it did not differ significantly between the singly and repeated exposed groups of rats or mice. Per kg body weight, mice were observed to metabolize about 5-fold more MC than did rats. Immediately after the end of the final 6-hour exposure, ^{14}C-activity was found in the liver, kidney, and adipose tissue. After 72 hours postexposure, levels in all three sites decreased appreciably; activity was not detectable in adipose tissue of mice nor in liver of rats. Prior repeated exposure to MC did not significantly alter the concentration of ^{14}C-activity in any tissue of either species.

When data obtained in the study were compared to similar data obtained during the course of exposure to younger rodents (Schumann et al., 1982a), an apparent age-related difference in metabolism was noted. The apparent effect was greater in mice; there was a 2-3 fold increase in body burden and a 5-6 fold greater extent of metabolism of MC in the 18-month-old mice compared to the 2.5 to 3.5-month-old mice that had been exposed to 1,500 ppm (8,100 mg/m^3) ^{14}C-MC in only a single 6-hour period. One factor that could account for the increased body burden and metabolism is a slower rate of pulmonary clearance in the older animals. The older rats cleared about 50 percent less ^{14}C-MC during the first three hours after exposure relative to younger rats (Schumann et al., 1982a); the difference was not as great in mice.

As previously discussed man appears to possess a very limited capacity for metabolism of MC. Table 4-6 summarizes data taken from several investigative studies showing the amount of urinary metabolites excreted after exposure to various concentrations (4.3 to 213 ppm; 23.2 to 1,150 mg/m^3) of MC in inspired air. Seki et al. (1975) surveyed workers in 4 printing plants where MC was the sole organic solvent in use. The workers were exposed 8 hours daily, 5 1/2 days per week, over a period of at least 5 years. Urine samples were collected in the latter half of the work week. Their data in Table 4-6 show a proportional increase of both TCE (as glucuronide) and TCA, and also total trichlorinated compounds (TTC) with increased air concentrations of MC. Seki et al. expressed their data as a linear relationship between inspired MC concentration and urinary metabolite excretion, with an added constant increment presumably due to continued excretion from the previous day's exposure. From this observation and from evidence of continued excretion during exposure-free weekends, they suggested that MC accumulates in the body up to a steady-state body burden defined by the air concentration of each daily exposure. Seki et al. also noted that the amounts of metabolites excreted daily in the urine of these workers were less than 5 percent of that observed with comparable exposures to trichloroethylene, a compound that Monster (1979) has shown to be metabolized from 60 to 80 percent to TCE and TCA. This comparison suggests that about 5 percent or less MC is metabolized.

The data (Table 4-6) of Monster et al. (1979b) and of Humbert and Fernandez (1977) were obtained from subjects given single 4-hr and 8-hr exposures to 70 and 145 ppm (378 and 783 mg/m^3), and 72 and 213 ppm (389 and 1,150 mg/m^3) MC, respectively. Total urinary excretion of TCE (glucuronide) and TCA is observed to be proportional to the inspired air concentration of MC and to the expected body burden from these exposures (with duration of exposure also taken into account). Both these research groups attempted a balance study by estimating the retained body dose of MC either by measuring lung clearance during exposure (Monster et al., 1979b), or by integrating over the MC alveolar air decay curve to infinite time postexposure (Humbert and Fernandez, 1977). The percentages of the retained dose metabolized to TCE and TCA were 2.5 and 6.3 percent, respectively. The difference can be ascribed to the different methodologies used for estimating body dose and their inherent imprecisions. In both studies, the percentages of MC metabolized were found to be independent of the retained dose (Table 4-6). A similar observation with rats was made by

TABLE 4-6. RELATION BETWEEN INHALATION EXPOSURE AND URINARY METABOLITES OF MC

Seki et al., 1975

Average concentration of metabolites in urine samples from workers daily exposed to MC.

Plant Plant	Air conc. MC, ppm	No. Subjects	TCE*	TCA	TTC/g creatinine
			mg/ℓ	mg/ℓ	
A	0	30	0	0	0
B	4.3	10	1.2	0.6	2.1
C	24.6	26	5.5	2.4	6.8
D	53.4	10	9.9	3.6	15.0

Monster et al., 1979b

Averaged amounts of metabolites in total urine collected 70-hr post single exposure (4 hr).

Air Conc. MC, ppm	No. Subjects	TCE* mg	TCA mg
72	6	5.5	1.5
145	6	11.5	2.8
Estimated as % retained dose		2%	0.5%

Humbert and Fernandez, 1977

Averaged amounts of metabolites in total urine collected for 12 days post single exposure (8 hr).

Air cond. MC, ppm	No. Subjects	TCE* mg	TCA mg
72	3	15.2	5.2
213	2	30.7	13.0
Estimated as % retained dose		4.6%	1.7%

*TCE found as glucuronide.

Eben and Kimmerle (1974), who measured urinary excretion of TCE and TCA for 3 days after a 4-hr inhalation of MC at 221 and 443 ppm (1,193 and 2,392 mg/m^3). The amounts of the metabolites excreted were proportional to the inspired concentration of MC. A 1.7-fold increase in the quantity of urinary metabolites was measured with the 2-fold range of exposure levels. In rats, the urinary ratio of TCE/TCA was 20/1 rather than the 3/1 observed in humans (Table 4-6).

Nolan et al. (1983) in a report submitted for publication found that 5 to 6% of the amount of MC inhaled by volunteers during a 6-hour exposure was excreted in urine as TCE and TCA. The amounts were reported to be extremely variable, thus indicating that such measurements provide at best only a rough estimate of exposure.

Caperos et al., (1982) have developed a kinetic model simulating occupational exposures and one which may be suitable for biologic monitering. This model was reported to be consistent with the experimental data of Humbert and Fernandez (1977). The results of the simulation of varying workday exposures suggested that TCE is the most sensitive and representative indicator of exposure; fluctuatuions in exposure concentration had a marked influence upon urinary TCE concentrations, but no effect upon urinary TCA.

A controlled human exposure study designed to determine if simultaneous measurement of MC and Pco_2 in alveolar air represents a useful technique by which MC exposures can be monitored was conducted by Guillemin and Gubéran (1982). A linear relationship between MC levels and Pco_2 in alveolar air samples was demonstrated. Both MC and Pco_2 levels varied, depending on the technique used to collect alveolar air samples and the extent to which the eight volunteers hyper- or hypoventilated. Analysis of variance for those tests that used a "standard" sampling technique showed an average coefficient of variation of 5.6 percent. During this series, the sample was collected during the last part of a prolonged (but not forced) expiration following a normal inspiration. All 8 volunteers were exposed, at rest, 4 hours both in the morning and afternoon to MC ranging in concentration from 203 to 236 ppm (1,096 to 1,274 mg/m^3). Alveolar sampling was performed on the first post-exposure day between 8 and 12 a.m. The results demonstrated that MC and Pco_2 should be corrected for hyper- and hypoventilation and for dilution of alveolar air with dead space air by a proportional adjustment of MC concentration at the mean normal alveolar Pco_2 or by disregarding the samples with a Pco_2 outside the normal range.

A reasonable conclusion from these studies is that MC is minimally metabolized by man on the order of 3 to 6 percent of the inhaled dose. The percentage of the dose metabolized is constant. Due to the limited extent of metabolism of MC and the observations that metabolism in rats and mice is saturated at high concentrations, saturation of metabolism does not appear to play a significant role in the pharmacokinetics of MC during likely human exposure situations.

4.2.2 Kinetics of Blood and Urine Metabolites

The blood and urinary metabolites of MC (total amount, excretion ratios, and excretion time-course) are of interest as quantitative indices of exposure and body burden. However, the known metabolites of MC--TCE, TCE-glucuronide, and TCA -- are not pathognomonic of MC, but are also metabolites of other chlorinated hydrocarbons, e.g., trichloroethylene.

From studies of men exposed to 70 and 145 ppm (378 and 783 mg/m^3) MC inhaled for 4 hours, Monster et al. (1979b) found that the TCE concentration in blood was proportional to both the inspired concentration and the blood concentration of MC. TCE blood concentration was about 4 percent that of MC [0.2 mg ℓ^{-1} and 0.09 mg ℓ^{-1} TCE for 145 and 70 ppm (783 and 378 mg/m^3) MC inspired, respectively]. After termination of exposure, blood TCE concentration declined exponentially with a half-life of 10 to 12 hours. Urinary appearance of TCE and TCE-glucuronide paralleled the disappearance of blood TCE, and daily excretion decreased with a half-time of renal elimination of 10 to 12 hours. This value is in agreement with that observed after ingestion of TCE itself (Briemer et al., 1974; Muller et al., 1974). In contrast to the first-order blood decay kinetics of TCE, blood concentrations of TCA progressively increased after the end of MC exposure for about 40 hours before declining exponentially with a half-life of 70 to 85 hours. Consequently, TCA appeared in the urine in almost equal daily amounts for 3 days before decreasing. Exogenous TCA administered to men has a similarly long half-time of renal elimination of 50 to 82 hours, presumably because of very tight non-covalent binding to plasma proteins (Paykoc and Powell, 1945; Muller et al., 1974). Therefore, the rise in plasma concentration of TCA during the 24 to 48 hours period following exposure is due to a rate of formation of TCA from TCE greater than the rate of renal elimination of TCA. Similar observations on daily urinary excretion of TCE, TCE-glucuronide, and TCA following acute MC inhalation exposure have been made in man by Humbert and Fernandez (1977), and in rats by Eben and Kimmerle (1974).

Stewart et al. (1969) investigated urinary metabolite excretion in men repeatedly exposed to MC (500 ppm; 2,700 mg/m^3), 7 hours per day for 5 days.

The daily ratio TCE/TCA during exposure remained relatively constant (about 2.8), but rapidly decreased within several days after exposure (5th day, 0.4), indicating that the daily urinary TCE excretion decreased while the TCA excretion decreased at a lesser rate or may have gradually increased. Eben and Kimmerle (1974) also chronically exposed rats for 14 weeks (8 hours daily, 5 days per week) and measured blood levels of MC, TCE, and TCA, as well as daily urinary excretion. Blood concentrations of MC and TCE (determined immediately after daily exposure) remained essentially constant during the entire 14 week period, with a concentration ratio of MC:TCE of 10:1. Weekly urinary excretion of TCE reached a plateau within 3 to 4 wk, whereas TCA weekly excretion was constant throughout the entire 14-wk period. The urinary excretion ratio TCE/TCA was thus initially low, but after 3 to 4 weeks exposure reached a constant value of approximately 18.

These findings show that the daily urinary ratio of TCE/TCA (mg/day) during chronic MC exposure of both man and rats is determined by the relative rates of metabolic formation and by the differences in renal elimination of TCE (rapid, half-time 10 to 12 hours) and TCA (slow, half-time 50 to 70 hours). In blood, both metabolites achieve steady-state plateau concentrations (TCE more rapidly than TCA) related to the inspired air concentration of MC and its metabolites. In urine, the TCE/TCA ratio, initially low, rises to a constant daily value. After termination of either acute or chronic exposure, the ratio of the amounts of daily urinary metabolites, TCE/TCA (mg/day), is highest 24 hours after exposure. Thereafter, it progressively decreases daily to less than unity by the 5th or 6th day postexposure because of the relatively rapid renal elimination of TCE and the slow renal elimination of TCA (Stewart et al., 1969; Eben and Kimmerle, 1974; Monster, 1979).

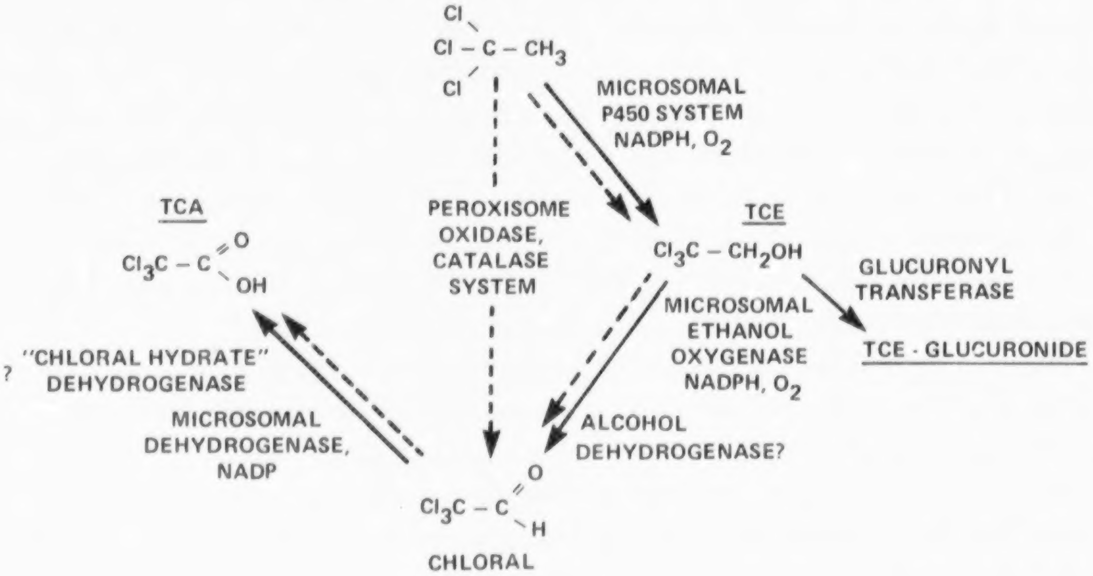
The profile of TCE and TCA urinary excretion during and after MC exposure is similar to that observed for trichloroethylene (Nomiyama and Nomiyama, 1971; Muller et al., 1974; Sato et al., 1977; Monster et al., 1976, 1979a; Fernandez et al., 1977). However, the ratio of TCE/TCA excreted is 2-fold greater for MC than for trichloroethylene, suggesting differences in the pathways and rates of metabolism to TCE and TCA for these two chlorinated hydrocarbons. Trichloroethylene, a chlorinated olefin, is thought to be metabolized by hepatic microsomes to an epoxide, then to chloral hydrate, and thence to TCE and TCA (Liebman and Ortiz, 1977; Henschler, 1977; Van Duuren, 1977). However, neither epoxide nor chloral hydrate have been identified as metabolites of MC. Comparison of the percentage of the body dose metabolized to TCE and TCA (MC, 3-6 percent vs trichloroethylene, 80-90 percent) explains the 25- to 30-fold difference in the total amounts of these metabolites (mg/day) excreted into urine for comparable body doses retained after inhalation exposure (Seki et al., 1975; Stewart, 1968; Stewart et al., 1969; Monster, 1979; Ikeda and Ohtsuji, 1972; Ikeda et al., 1972). In a comparative study of rats exposed to 200 ppm (1,080 mg/m^3) MC or its 1,1,2-isomer, Ikeda and Ohtsuji (1972) reported that the urinary excretion of total chlorinated metabolites was less for the 1,1,2-isomer, indicating that the 1,1,2-isomer is metabolized to an even smaller extent than MC. These workers determined the urinary metabolites of the 1,1,2-isomer as TCE and TCA by a spectrophotometric method based on the Fujiwara reaction. However, the MC isomer requires a shift of a chlorine atom from one carbon to the other in order to form TCA or TCE, an unlikely reaction *in vivo* for a saturated aliphatic, and it is probable that dichlorometabolites were actually measured. Yllner (1971a) injected ^{14}C-labeled 1,1,2-trichloroethane, i.p., into mice. Over a 3-day period, 73 to 87 percent of the activity was recovered in the urine, less than 2 percent in the feces. Expired air contained 16 to 22 percent of the radioactivity (60 percent $^{14}CO_2$ and 40 percent unchanged parent compound). From 1 to 3 percent remained in the animal. This indicates that the metabolism of 1,1,2-trichloroethane is significantly greater than MC.

The interactions of MC and tetrachloroethylene upon their metabolism is a focus of the research investigations of Ikeda and coworkers. An 8-hour exposure of male Wistar rats to a mixture of these compounds at their TLV® levels was reported to cause a statistically significant ($p < 0.01$) decrease in TCE, the principal urinary metabolite of MC. In the opinion of the authors, this apparent suppression of MC metabolism by tetrachloroethylene is most likely a result of suppression of oxidation of MC to TCE (Koizumi et al., 1982). *In vitro* kinetic studies are in progress.

4.2.3 Enzyme Pathways of Methyl Chloroform Metabolism

The metabolic pathways and enzyme mechanisms for the metabolism of halogenated hydrocarbons assume considerable importance for understanding and assessing cellular toxicity. Compounds that in the course of their metabolism form intermediates reactive with cellular macromolecules, e.g., epoxides or free radicals, are associated with enhanced cellular toxicity and carcinogenic potential (Van Duuren, 1977). In comparison with other halogenated hydrocarbons, MC is not extensively metabolized by mammalian systems; this may explain, in part, its lower toxicity and carcinogenic potential (Weisburger, 1977).

Figure 4-3 summarizes the presently postulated enzyme steps in the biotransformation of MC to TCE and TCA, the only known metabolites appearing in the plasma and urine of animals and man (Table 4-6). It is assumed that metabolism occurs principally in the liver, although *in vitro* experiments that directly demonstrate and evaluate MC metabolism by the liver have only been reported for TCE production (Ivanetich and Van Den Honert, 1981).



MICROSOMAL P450 SYSTEM NADPH, O2
PEROXISOME OXIDASE, CATALASE SYSTEM
TCA
TCE
GLUCURONYL TRANSFERASE
TCE · GLUCURONIDE
MICROSOMAL ETHANOL OXYGENASE NADPH, O2
? "CHLORAL HYDRATE" DEHYDROGENASE
MICROSOMAL DEHYDROGENASE, NADP
ALCOHOL DEHYDROGENASE?
CHLORAL

Figure 4-3. Postulated pathways of hepatic biotransformation of MC.

Source: Davidson (1980) and Ivanetich and Van Den Honert (1981).

Schumann et al. (1982a), using ^{14}C-MC studied the routes of elimination in both rats and mice. Hake et al. (1960), found that about 0.5 percent of the dose (of the 2 percent metabolized; see Table 4-7) was converted to $^{14}CO_2$, following 1,1,1-trichloroethane-1-^{14}C administration (intraperitoneal) to rats, suggesting carbon-carbon cleavage. The origin of the $^{14}CO_2$ is an open question since both Schumann et al. (1982a) and Hake et al. did not identify the source. The ^{14}C-MC used by Hake et al. was contaminated with trace amounts of the MC isomer 1,1,2-trichloroethane-1-^{14}C (0.4 percent) and 1,1-dichloroethane-1-^{14}C (0.5 percent). Both of these compounds were reported by Van Dyke and Wineman (1971) and by Van Dyke (1977) to be readily dechlorinated by the rat liver microsomal P450 system. The optimal configuration for dechlorination was a dichloromethyl group. Hence, 1,1,2-trichloroethane, the more toxic isomer, was readily dechlorinated but 1,1,1-trichloroethane was not. For the isomer 1,1,2-trichloroethane, the products were identified as mono-and dichloroethanol and mono- and dichloroacetic acid (Van Dyke and Wineman, 1971), which are known to be metabolized to CO_2 or to form glutathione conjugates (Yllner, 1971a,b). Carlson (1973) found that pretreatment of rats with phenobarbital, but not methylcholanthrene, potentiated hepatotoxicity (measured as serum SGOT and SGPT) of both inhaled MC and its 1,1,2-isomer, but the toxicity of the isomer was increased to a far greater extent. These several observations suggest that 1,1,2-trichloroethane, but not MC, may be extensively metabolized by the oxidative dechlorination system of Van Dyke and Wineman (1971).

Figure 4-3 proposes that the initial pathway of MC transformation is hydroxylation of MC to TCE by the microsomal P450 mixed function oxidase system (Ivanetich and Van Den Honert, 1981).

This view is supported by the observations of Cox et al. (1976) and Pelkonen and Vainio (1975) in which a P450 type I binding spectrum was demonstrated upon aerobic incubation of MC with rat liver microsomes plus NADPH. Apparent induction of the P450 drug metabolizing system was observed in the experiments of Fuller et al. (1970) and Lal and Shah (1970). Lal and Shah (1970) exposed Swiss albino, random-bred male mice to 3,000 ppm (16,200 mg/m^3) MC for either 24 hours or for 4 or 8 hours/day for several days. Hexobarbital (80 mg/kg), sodium barbital (275 mg/kg) or chloral hydrate (350 mg/kg) induced sleeping time was measured at predetermined times after exposure. The 24-hour exposure produced a maximum reduction in the duration of hexobarbital sleeping time but had no effect when either barbital or chloral hydrate was used.

The significance of the difference of means between control and experimental groups was determined by the Wilcoxon Rank Sum Test. Where the 24-hour exposure was completed in 3 to 6 exposure periods, each separated by 18 to 21 hours, MC inhalation showed a statistically significant cumulative effect. A single 8-hour exposure was ineffective.

Fuller et al. (1970) extended these observations by investigating the inductive effect of MC on hepatic drug metabolism. Male Sprague-Dawley rats and Swiss albino, random-bred mice were exposed to MC (2,500 to 3,000 ppm; 13,500 to 16, 200 mg/m^3) in an air flow-regulated chamber for 24 hours. Pretreatment with inhibitors of protein synthesis (cyclohexamide and actinomycin D) were used in an attempt to block the effect of MC on the hepatic drug-metabolizing system. Inhalation of MC by untreated rats decreased the duration of action of hexobarbital, meprobamate, and zoxazolamine. This was accompanied by an increase in the metabolism of hexobarbital, zoxazolamine and aminopyrine _in vitro_ by hepatic microsomal enzymes. Cytochrome P-450 and cytochrome c reductase were also increased. Pretreatment with cyclohexamide and actinomycin D prevented the MC-induced decrease in hexobarbital sleeping time and the increase in drug metabolism. The authors suggested that the results indicate that the increase in drug metabolism after MC inhalation is related to induction of new enzyme protein. It was further suggested that this offers a sensitive prepathologic measure of MC toxicity since induction occurs after exposure to concentrations of MC that do not produce histologically detectable liver lesions.

These observations raised the possibility that MC might induce its own metabolism with repeated daily exposure. However, a number of observations by others indicate that this is not the case. Platt and Cockrill (1969) reported MC to be essentially without effect in enhancing the hepatic MFO system of mice orally dosed with 1,650 mg/kg/day for 7 days. Savolainen et al. (1977) did observe increased P-450 content above controls at 3 hours of exposure of male Sprague-Dawley rats to 500 ppm (2,700 mg/m^3) but upon cumulative exposure of rats for 5 days, P-450 decreased below control values. This decrease is consistent with the observations of Vainio et al. (1976) who treated animals intraperitoneally. As previously discussed in this chapter, Schumann et al. (1982b) found that repeated exposure of rats and mice to 1,500 ppm (8,100 mg/m^3) for 16 months did not significantly alter the disposition of MC compared to singly-exposed rats and mice.

In contrast to the results observed earlier by Lal and Shah (1970), Shah and Lal (1976) found that when MC was administered to Swiss-albino, random-bred mice by i.p. injection, a potentiation of pentobarbital sleeping time and a reduction in the metabolism of hexobarbital by liver microsomes were observed. When MC was topically applied, pentobarbital sleeping time was reduced by 36 percent, similar in action to that observed previously by inhalation. Similar results had been reported earlier by Plaa et al. (1958) in male albino mice of the Princeton strain when MC was administered subcutaneously. MC significantly ($p = 0.01$) potentiated sleeping time induced by pentobarbital. These observations may be explained on the basis of different amounts of MC reaching the liver by various routes and methods of administration.

The origin of TCA as a plasma and urinary metabolite of MC is postulated to occur from enzymic oxidation of TCE. TCE-glucuronide, also present in plasma and urine, is presumed to be formed by glucuronyl transferase. TCE, exogenously administered to man, yields TCA as a metabolite (Marshall and Owens, 1954; Owens and Marshall, 1955a,b; Muller et al., 1974). Marshall and Owens (1954) also observed TCA formation during TCE incubation with rat or dog liver slices. The most likely reaction for the oxidation of TCE to TCA would involve the enzyme alcohol dehydrogenase (Figure 4-3). However, *in vitro* studies with alcohol dehydrogenase purified from horse liver, and with rat liver cytosol fractions, have shown that TCE is a poor substrate for alcohol dehydrogenase and that significant conversion to trichloroacetaldehyde (chloral) does not occur (Sellers et al., 1972; Friedman and Cooper, 1960; Marshall and Owens, 1954). The reverse reaction --reduction of chloral to TCE -- proceeds rapidly with a K_m of 2.7×10^{-3}M for horse liver enzyme (Butler, 1948, 1949; Marshall and Owens, 1954; Owens and Marshall, 1955a,b; Friedman and Cooper, 1960; Sellers et al., 1972). Also, chloral hydrate has been sought, but not detected, as an intermediate metabolite in plasma of rats and man exposed by inhalation to MC (Monster et al., 1979a; Eben and Kimmerle, 1974). On the other hand, chloral hydrate exogenously administered is very rapidly metabolized *in vivo* with a half-life of only a few minutes, yielding both TCE and TCA as metabolites in plasma and urine (Butler, 1948; Marshall and Owens, 1954; Owens and Marshall, 1955a,b; Breimer et al., 1974; Muller et al., 1974; Cole et al., 1975).

The conversion of chloral to TCA is usually ascribed to so-called "chloral hydrate dehydrogenase," a substrate-specific NAD-dependent enzyme described by Cooper and Friedman (1958). This enzyme was obtained from rabbit liver acetone powders. Cooper and Friedman reported that acetaldehyde was not a substrate, but rather a markedly effective inhibitor; they did not demonstrate a normal endogenous substrate. Human cytosolic acetaldehyde dehydrogenase does not convert chloral hydrate to TCA (Kraemer and Deitrich, 1968; Blair and Bodley, 1969; Sellers et al., 1972). Grunnet (1973) reported that chloral hydrate is not a substrate for mitochondrial NAD-dependent acetaldehyde dehydrogenase. Microsomal NADP-dependent acetaldehyde dehydrogenases with broad substrate specificity have been described, but whether chloral hydrate is a substrate has not been determined (Tottmar et al., 1973).

There is little evidence which precisely defines the enzymatic pathways for the metabolism of MC to TCE by P450 mixed function oxidase system, and TCE to TCA by classical alcohol dehydrogenase and acetaldehyde dehydrogenase enzymes. Although as yet uninvestigated, it is possible that TCE may be a substrate for the microsomal P450 ethanol oxidative system (MEOS) described by Lieber and his coworkers for ethanol and other alcohols (Lieber and De Carli, 1968; Teschke et al., 1977). TCE has been shown by Uehleke et al. (1976) to give a binding spectrum with rat microsomes. An alternative pathway also uninvestigated is afforded by the peroxisomal oxidase-catalase system (Figure 4-3), which is known to readily oxidize ethanol and a broad spectrum of other substrates (Masters and Holmes, 1979; Chance et al., 1977). Indeed, peroxisomes may perhaps be involved in the initial oxidation of MC to TCE and other halogenated hydrocarbons as well.

4.3 SUMMARY AND CONCLUSIONS

Like other solvents of this group, inhalation and lung absorption of MC vapor in the air is the most important and rapid route of absorption into the body. Absorption through the skin by direct liquid contact is slow. At the accepted TWA value (350 ppm; 1,890 mg/m^3) for an 8-hr exposure, less than 2 g may be expected to be absorbed into the body of a normal 70 kg man. This is because pulmonary absorption is directly related to the blood/air partition coefficient, which for MC is less than that of most other structurally related solvents. MC total body dose increases in direct proportion to inspired air concentration until steady-state is reached; it is also increased by physical activity during exposure. MC distributes throughout the body, readily crossing the blood-brain barrier and possibly the placental barrier as well. It can be assumed that MC may also distribute into the colostrum or milk of nursing mothers, although no specific data are available. Relative body tissue concentrations are not known, but there is a high affinity for adipose tissue due to the higher lipid/ blood partition coefficient of MC compared with that of other related solvents. Blood and tissue concentrations achieved at equilibrium are directly proportional to inspired air concentration.

MC is metabolized in man to a very limited extent--no more than 6 percent of the total body dose. Metabolism appears to occur principally in the liver, and to an unknown extent in other tissues, yielding only trichloroethanol (TCE) and trichloroacetic acid (TCA) as identified metabolites. Urinary excretion of these metabolites is proportional to inspired air concentration and the total body dose of MC at low exposure concentrations. At high concentrations (1500 ppm), the biotransformation of MC appears to be a saturable, dose-dependent process. However, saturability is of little practical significance since metabolism occurs to a limited extent and ambient air concentrations are orders of magnitude lower than those associated with metabolic saturation. Detailed information concerning the enzymatic pathways of metabolism is lacking. The mechanism(s) of the initial biotransformation of MC to TCE are speculative.

During post-exposure, between 80 to 90 percent of MC is excreted unchanged by the lungs. Alveolar air concentration and blood concentration decline in a parallel exponential fashion exhibiting three major components of elimination with half-times of approximately 1, 9, and 30 hours. The long half-time of elimination (30 hours) is related to elimination from adipose tissue.

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5. TOXIC EFFECTS

Since its commercial introduction in 1954, methyl chloroform has been used increasingly as an industrial solvent and in consumer products. Usually the most important path of entry into the body is by inhalation. The principal health effect of over-exposure involves the central nervous system. While thresholds for such effects are difficult to characterize, levels around 1,000 ppm (5,400 $\mu g/m^3$) may result in coordination problems. At much higher levels, anesthesia becomes apparent and death may occur at 1 to 3 percent due to anesthesia and/or cardiac toxicity. Unlike other chlorinated hydrocarbons, MC has not been associated with clearly evident liver or kidney damage. The available literature on the possible toxic effects of MC is reviewed in this chapter.

5.1 HEALTH EFFECTS IN HUMANS

5.1.1 Experimental Studies

Experimental studies in humans have centered around three general areas: (1) clinical experiences with MC as an anesthetic; (2) the kinetics of MC absorption and excretion after exposure via the inhalation and cutaneous routes; and (3) the impairment of psychophysiological functions in humans exposed to MC.

Dornette and Jones (1960) administered MC to 50 patients undergoing elective surgery. Nitrous oxide-oxygen (4:1) was used as the vehicle and as a supplemental anesthetic agent. The concentration of MC required for induction of surgical plane anesthesia varied from 10,000 to 26,000 ppm (54,000 to 140,400 mg/m^3), and for maintenance of light anesthesia from 6,000 to 22,500 ppm (32,400 to 121,500 mg/m^3). Rapid induction and recovery, analgesia, and the absence of disagreeable odor, respiratory depression, postoperative depression, nausea and vomiting were listed as advantages in the use of MC. A definite disadvantage was depression of blood pressure during anesthesia of moderate depth. The tendency to develop ventricular arrhythmias during hypoxia was also observed but this effect was reversed when effective oxygenation was reestablished.

Cardiac sensitization from exposure to high concentrations of halogenated hydrocarbons, with resultant increased susceptibility of the heart to catecholamine-produced arrhythmias, e.g., ventricular fibrillation or ventricular tachycardia, is a well-known phenomenon. This topic has been comprehensively reviewed by Aviado et al (1976). Clark and Tinston (1973) believe that cardiac sensitization caused by these relatively inert, lipid soluble hydrocarbons is very likely to be a structurally non-specific action on myocardial membranes by solution and distribution within these membranes, and is, therefore, an example of "physical toxicity" (see Sect. 5.2.3). From an evaluation of 14 halogenated hydrocarbons in dogs, Clark and Tinston (1973) found that the cardiac sensitizing potencies, as determined by the partial pressure in inhaled air needed to sensitize the heart to epinephrine, was directly related to their saturation vapor pressures. In a similar way, the anesthetic potency or narcotic action of the halogenated hydrocarbons, a structurally nonspecific action, is directly related to the blood/air or lipid/air partition coefficient for these compounds (Table 4-1) (Miller et al., 1972; Eger et al., 1965; Sato and Nakajima, 1979).

TABLE 5-1. SUBJECTIVE AND PHYSIOLOGICAL RESPONSES TO A CONSTANTLY INCREASING METHYL CHLOROFORM VAPOR CONCENTRATION OVER A PERIOD OF 15 MINUTES

Concentration (ppm)	Responses to Exposure
0 to 1000	Increasing awareness of a slightly sweet, not unpleasant odor.
1000 to 1100	Mild eye irritation noted in 6 of 7 subjects.
1900 to 2000	6 of 7 subjects aware of throat irritation.
2600	1 subject very lightheaded.
2650	2 subjects unable to stand. 3 subjects very lightheaded, but able to stand. 2 subjects were not lightheaded, and one of these was able to demonstrate a normal Romberg test.

Source: Stewart et al. (1961).

Siebecker et al. (1960) reported that electroencephalographic patterns during MC anesthesia showed little change before circulatory depression and that the changes were similar to those during halothane anesthesia. The investigators found MC to be less potent clinically than either chloroform or halothane in supplementing nitrous oxide-oxygen for anesthesia.

Stewart et al. (1961) evaluated the acute effects of increasing concentrations (0 to 2,600 ppm; 0 to 14,040 mg/m^3) of MC over a 15-minute exposure period. A commercial grade of MC was used. Table 5-1 shows the subjective and physiological responses during exposure to MC. In another experiment in which 6 subjects were exposed to MC at 500 ppm (2,700 mg/m^3) for 78 minutes or 186 minutes, no eye irritations or dizziness occurred, nor were balance or coordination affected. Exposure at 900 to 955 ppm (4,860 and 5,157 mg/m^3) for 73, 35, and 20 minutes produced a number of psychophysiological effects which are listed in Table 5-2.

TABLE 5-2. SUBJECTIVE AND PHYSIOLOGICAL RESPONSES TO METHYL CHLOROFORM VAPOR CONCENTRATIONS OF 900 TO 1000 PPM

Average Concentration	Responses to Exposure[a]
900 ppm for 20 minutes (3 subjects)	Positive Romberg in one subject. Greater effort required to perform a normal Romberg in two subjects after 10 minutes of exposure. Heel-to-toe walking normal. Two subjects experienced lightheadedness above 900 ppm.
910 ppm for 35 minutes (2 subjects)	Greater mental efforts required to perform a normal Romberg test after 10 minutes of exposure. All heel-to-toe walking performed well. One subject experienced persistent lightheadedness above 900 ppm.
951 ppm for 73 minutes (3 subjects)	Greater mental effort required to perform a normal Romberg test after 10 minutes of exposure. After 15 minutes of exposure one subject had consistently positive Romberg. Heel-to-toe walking performed well by all during exposure. No lightheadedness.

[a] Mild eye irritation was noted by all subjects when vapor concentrations rose above 1000 ppm.

Source: Stewart et al. (1961).

In another study, Torkelson et al. (1958) exposed humans to MC. Exposure to 550 ppm (2,970 mg/m^3) for 90 minutes had no measureable effect on the vital signs being monitored. Exposure to 500 ppm (2,700 mg/m^3) for 450 minutes produced no significant changes in pulse, respiration, blood pressure, reflexes, or equilibrium; liver function tests were also negative. Exposure to 1,000 ppm (5,400 mg/m^3) for 30 minutes was also without effect. However, exposure to 900 to 1,000 ppm (4,860 to 5,400 mg/m^3) for 75 minutes resulted in slight eye irritation and a feeling of lightheadedness, and the Flanagan and Romberg tests revealed a slight but definite loss of coordination and equilibrium. ECG and liver function tests were normal. Exposure to 1,900 ppm (10,260 mg/m^3) for 5 minutes resulted in an obvious disturbance of equilibrium and an abnormal Romberg test.

In 1969, Stewart et al. reported that exposure to 500 ppm (2,700 mg/m^3) for periods of 6.5 to 7 hours/day for 5 consecutive days resulted in mild subjective responses (sleepiness, eye irritation, and mild headache). The only untoward physiological response was an abnormal Romberg test. However, the two individuals who had the abnormal response had, according to the authors, demonstrated difficulty performing the test before any exposure. None of the clinical tests performed during or following the exposure was abnormal.

Twelve subjects were exposed to 250, 350, 450, and 550 ppm (1,350; 1,890; 2,430; and 2,970 mg/m^3) of MC in inspired air during four continuous 30 minute periods in an experiment reported by Gamberale and Hultengren (1973). The air-gas mixture was supplied via a breathing valve and a mouthpiece with very low resistance. The effects of the introduction of the breathing tube were not assessed. The presence or absence of MC was reportedly disguised by the use of menthol crystals. In the final 20 minutes of each exposure period, five performance tests were made. Two of the tests were of perceptual speed and the others were tests of simple reaction time, choice reaction time, and manual dexterity. The same subjects were also studied under control conditions in which inspired air contained no MC but in which all operations and measurements were the same as during exposure to the solvent. To balance the training effects between experimental and control conditions, the order of conditions was reversed for half the subjects.

The change in mean performance level during exposure to the increasing concentrations of MC differed systematically from the change in performance under control conditions. The level of performance in the manual dexterity test and two perceptual tests were affected by training; however, the training effect was less pronounced during exposure to MC. The tests of reaction time were less sensitive to training and, with these, there was an absolute decline in performance capability as the exposure concentration increased. Statistically significant performance differences between experimental and control conditions were reported for all tests with exposure levels of 350 ppm (1,890 mg/m^3) or greater, but the subjects were tested repeatedly. Such testing usually yields more significant results than warranted (Benignus and Muller, 1982). This study contained several drawbacks: e.g., the substance used to disguise the MC odor may itself have had a toxic effect, and the introduction of a breathing tube may have induced stress in the subjects.

Salvini et al. (1971) evaluated psychophysiological effects after exposing six male university students in two groups of three to an average vapor concentration of 450 ppm (2,430 mg/m^3) MC for two periods of 4 hours, separated by a 1.5 hour interval. During exposure they alternated their activities with a one-hour study period followed by 20 minutes of physical excercise. Each subject was examined on two different days, four days apart. Each group alternated in the order in which they were exposed to the control and experimental atmospheres. The psychophysiological tests included a perception test with tachistoscopic (brief exposure to visual stimuli) presentation, the Wechsler Memory Scale test, a complex reaction time test, and a manual dexterity test. After two exposures, no disturbances in motor function, coordination, equilibrium, or behavior patterns were observed in any of the subjects. However, there were some complaints about eye irritation at peak concentrations when values ranged up to 500 ppm (2,700 μg/m^3). Under mental stress conditions, exposure to 450 ppm (2,430 mg/m^3) MC was reported to have "decreased perceptive capabilities". Mental fatigue was determined by comparing perception test results between the morning and afternoon tests. A small reduction in performance was also observed but was not statistically significant.

In 1975, Stewart et al. reported an experiment in which 20 individuals were exposed to 500 ppm (2,700 μg/m^3) or less for 7.5 hours per day, 5 days per week, for 3 weeks. No serious deleterious effects upon health or performance were detected and the health of the exposed individuals remained unimpaired during the inhalation studies. The blood chemistries, hematologies, urinalysis, electrocardiograms, and pulmonary function tests remained normal. Of the ten females exposed, nine reported that the odor of 350 ppm (1,890 μg/m^3) was objectionable. In contrast, none of the males objected to odor at any of the levels used. The authors stated that there was a lack of sleepiness and fatigue which had been reported by the subjects in the first repetitive study by Stewart et al. (1969). The authors suggested that monotony and boredom may have been the responsible factors in the first study. The authors stated further that "This study failed to corroborate the findings of Gamberale and Hultengren (1973) who reported that exposures to 1,1.1-trichloroethane at 350 ppm for 30 minutes impaired reaction time, perceptual speed and manual dexterity.... In our study, these cognitive tasks were not performed during the first 30 minutes but after several hours when the blood 1,1,1-trichloroethane was higher and when the decrement in performance should have been more pronounced ... our findings are in agreement with those reported by Salvini et al. (1971), who observed that exposure to 450 ppm for 4 hours failed to impair performance of a series of different psychophysiologic tests."

The effect of exposure to MC alone, and in combination with m-xylene, upon psychophysiological functions in humans was evaluated by Savolainen et al. (1981). Exposure of nine male volunteers to 200 and 400 ppm (1,080 and 2,160 mg/m^3) MC and to 400 ppm (2,160 mg/m^3) MC and 200 ppm m-xylene resulted in no marked effect upon reaction time, critical flicker fusion thresholds, or body balance. However, MC appeared to have a biphasic effect upon body balance. Exposure to MC at 200 ppm (1,080 mg/m^3) tended to decrease body sway whereas the higher exposure level had the opposite effect. No kinetic interactions between MC and m-xylene were observed. Savolainen and coworkers (1982) recently reported confirmation of the biphasic effect, using an identical protocol with the same subjects.

5.1.2 Occupational Studies

Chronic occupational exposure to other chlorinated solvents has occasionally been reported to cause adverse neurological and behavioral effects. Based on the absence of effects at 500 ppm (2,700 mg/m^3) in the better conducted human studies, relatively low level ambient exposures to MC would not be associated with adverse neurological and behavioral effects.

A recent attempt to assess the central and peripheral nervous system effects of MC in occupational situations was undertaken by Maroni and coworkers (1977). They studied a very small group (22 subjects) of female workers exposed to MC vapors at concentrations ranging from 110 to 990 ppm (594 to 5,346 mg/m^3). When compared to workers who were reportedly unexposed, no differences were found in clinical symptoms or measures of nerve conduction velocity and psychometric function.

In another study, Seki et al. (1975) surveyed 196 male workers employed in four Japanese printing factories where MC was the sole organic solvent in use. The four groups of workers were exposed to average concentrations of 4, 25, 28, and 53 ppm (22, 135, 151, and 286 mg/m^3). The workers participated in a medical interview coupled with a test for sense of vibration (studied at the distal joints of the thumbs and great toes using a 128 Hz tuning fork) as well as routine laboratory examinations, including peripheral hemograms, determination of blood specific gravity, and urinalysis for urobilinogen and protein. These examinations revealed no consistent dose-related adverse effects among the four groups of workers.

In the most recent study, Kramer et al. (1978) measured numerous physiological parameters of workers in two adjacent textile plants. Detailed blood chemistry and hematology studies were conducted for 151 matched pairs of employees to compare the exposed and unexposed partners. All employees in the exposed group had been exposed to MC (and other solvents), in varying concentrations, for up to 6 years. The concentration range was 11 to 838 ppm (59 to 4,525 mg/m^3), with a mean of 115 ppm (621 mg/m^3) MC. Of the 151 employees, 149 had been exposed for 12 to 60+ months. 135 out of 151 had TWA exposures of 50 to 149 ppm while 116 of 151 had a TWA exposure of 100 to 249 ppm. The control group was not exposed to chlorinated solvents.

Pairs were matched with regard to age, race, sex, work shift, job description and socioeconomic status, and examined within a 10 week period. Subject height, weight, blood pressure and pulse were obtained, and electrocardiograms were recorded. Laboratory blood determinations included hematocrit, hemoglobin, red blood cell count (RBC), white blood cell count (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) alkaline phosphatase, SGOT, SGPT, gamma glutamyl transpeptidase, total bilirubin, urea nitrogen, LDH, uric acid, total protein, A/G ratio, albumin, calcium, and phosphorus. For quantitative variables, t tests and tests of homogeneity of variance were made. Multiple regression analysis was performed on paired differences with respect to environmental variables and on the combined matched exposed and control populations with respect to demographic variables.

Breathing zone samples were collected in charcoal tubes, except in a few locations where area sampling was more practical, and analyzed on a portable gas chromatograph equipped with a flame ionization detector. Samples of expired air were analyzed immediately after collection by gas chromatography.

After explaining that some data were eliminated on the basis of subjects' smoking habits, high blood pressure, or prior illness, the authors presented statistical findings but no individual data. MC concentrations in the breath ranged from "less than 5 ppm" to "greater than 30 ppm," with the majority (127/151) between 5 and 29 ppm (27 and 157 mg/m^3). Comparison of the health test data between exposed and control subjects revealed no statistically significant differences except in a decrease in SGPT and a slight increase in albumin in the exposed group. The authors concluded that no health impairment was suffered by workers exposed to an average daily concentration of 115 ppm (621 mg/m^3) MC.

5.1.3 Accidental Exposure

Accidental exposure to excessive concentrations MC can lead to death. Table 5-3 lists the signs and symptoms of a number of cases in which the patients survived. These results suggest that MC has only a minimal potential for producing liver or kidney injury in man upon acute exposures. The primary toxic effect appears to be a reversible depression of the CNS, typical of an anesthetic agent. While abuse or misuse of any organic solvent has been difficult to control, the problem has diminished in recent years. The following is a review of some of the acute exposure incidents related to MC but where no exposure measurements were possible.

Two fatal cases in which the subjects intentionally inhaled cleaning fluids containing MC were reported by Hall and Hine (1966). A 19-year-old woman who was observed sniffing cleaning fluid over several days and acting irrationally was later found dead. Pathologic findings on autopsy were confined to the respiratory system, stomach and brain.

There were indications of chronic, intentional inhalation of a cleaning fluid containing MC in the other fatal case studied by Hall and Hine (1966). On autopsy, pathologic findings were confined to the respiratory system and the kidneys. In neither of these cases were drugs or solvents detected in the stomach contents, and no barbiturates were found in the blood. Blood levels of MC were 72.0 and 13.0 mg percent, respectively.

Twenty-nine cases of presumed sudden death from sniffing MC during 1964 to 1969 were reported by Bas (1970). These were among 110 cases of sudden death attributed to sniffing volatile hydrocarbons and halocarbons summarized by the author. In 18 of the 110 cases, death followed sniffing coupled with some form of exercise. No anatomical abnormalities were found from gross or microscopic postmortem examinations that could explain the sudden deaths. The author discussed the possibility that these deaths resulted from cardiac sensitization to endogenous catecholamines.

Six fatal cases were reported by Stahl et al. (1969). In the first case, a 20-year-old man was found dead in a closed space in which he had been working

TABLE 5-3. SIGNS AND SYMPTOMS OF PATIENTS SURVIVING INTOXICATION WITH METHYL CLHOROFORM

Reference	Patient	Amount	BP[a]	HR[b]	SGPT[c]	SGOT[d]	AP[e]	BUN[f]	ECG[g]	Comments
Stewart and Andrews, 1966	47 M	1 oz. orally	120/80	84	N[h]	N	NR[i]	N	N	No abnormal neurologic findings; proteinuria was 1+; hematocrit 53%; hemoglobin level 18 gm/100 ml
Stewart, 1971	44 WM	Inhalation	140/74	78	N	N	NR	N	N	Neurologic examination revealed abnormal Romberg's sign upon admission; hematocrit 46%; hemoglobin level 14.6 gm/100 ml
Stewart, 1971	55 WM	Inhalation	160/96	84	N	N	NR	N	N	No abnormal neurologic findings
Stewart, 1971	47 WM	Inhalation	160/90	76	N	N	N	N	N	No abnormal neurologic findings
Litt and Cohen, 1969	5 teenage M	Inhalation	NR	NR	NR	340-100 μ	7-30 μ	NR	NR	Nervous system symptoms including paresthesia, tinnitus, ataxia, and headache

[a] Blood pressure (mmHg)
[b] Heart rate (beats/min)
[c] Serum glutamic-pyruvic transaminase
[d] Serum glutamic-oxaloacetate transaminase
[e] Alkaline phosphatase
[f] Blood urea nitrogen
[g] Electrocardiogram
[h] N = normal
[i] NR = not reported

with a "paint remover." Gas chromatographic analysis revealed MC in all tissues. Upon autopsy, the lungs were found to be congested and moderately edamatous; the liver, spleen, kidneys, and brain were also congested. Microscopic examination of the brain suggested anoxia as the cause of death.

In the second case, a 17-year-old man was found dead in a room in which he was cleaning an air vent with MC. Gas chromatographic analysis revealed the presence of MC although blood levels were only 0.15 mg percent. The authors concluded that death was probably due to O_2 deprivation.

In the third case, an obese 24-year-old man was found dead in bed after having cleaned electrical equipment with MC. In the fourth, fifth, and sixth cases, three males were found dead in an unventilated 4 x 5 ft. compartment where they had been cleaning electrical equipment with MC. Blood levels of MC were 12.0 mg percent, 6.2 mg percent, and 6.0 mg percent in these victims.

Hatfield and Maykoski (1970) reported on a 27-year-old who was found dead in an aircraft tank that he had been cleaning with MC. The diagnosis was acute passive congestion of the viscera with petechial hemorrhages in the lung and brain. Hatfield and Maykoski estimated the concentration level of MC to which this worker was exposed at 62,000 ppm (334,800 mg/m^3).

Caplan et al. (1976) reported a fatal intoxication in which a 40-year-old female was apparently overcome while painting a bathroom. On external examination, the only significant abnormality was in the respiratory system. Histologically, the lungs showed acute edema and congestion, and the liver showed a mild fatty change. MC was identified in tissue samples.

Twenty-one deaths assumed to be related to the abuse or gross misuse of decongestant aerosol sprays containing MC in the solvent resulted in removal of several such products from the market (Federal Register 38:21935-36, 1973). As a followup, a Federal Register notice appeared in 1977 (Federal Register, 1977) and discussed this matter. It was stated: "The action concerning trichloroethane for inhalation is being taken because of the lack of safety and effectiveness data available on trichloroethane when it is used as a component of an aerosolized drug product intended to be inhaled by a sick person. The drug products containing trichloroethane implicated with 21 deaths were used as cough suppressants, primarily in infants."

5.2 EFFECTS ON ANIMALS

5.2.1 Acute and Subacute Effects

The LD_{50}'s of MC for various species are found in Table 5-4. Administration of single oral doses yielded LD_{50}'s for laboratory animals ranging from 8.6 gm/kg for guinea pigs to 14.3 gm/kg for rats (Torkelson et al., 1958). The solvent caused slight transitory irritation in the eyes. The toxicity from skin absorption was found to be low, as doses of 4 gm/kg failed to kill any rabbits exposed for 24 hours. Even 15.8 gm/kg failed to kill all rabbits tested with undiluted liquid MC, applied under a cuff for 24 hours. Repeated multiple daily application of 500 mg/kg to the skin of rabbits caused no effect other than reversible local irritation. In other experiments, laboratory animals were exposed repeatedly to 500, 1000, 2000, and 10,000 ppm (2,700; 5,400; 10,800; and 54,000 mg/m^3) in order to establish conditions safe for repeated exposure. Rats, guinea pigs, rabbits, and monkeys were unaffected after 6 months of repeated 7 hour exposures 5 days/week to 500 ppm (2,700 mg/m^3). Female guinea pigs, which were found to be the most sensitive in previous experiments, were able to tolerate 1000 ppm (5,400 mg/m^3) for 0.6 hour/day for 3 months and 2000 ppm (10,800 mg/m^3) for 0.1 hour/day with no detectable adverse effects. Male rats tolerated exposure to 10,000 ppm (54,000 mg/m^3) for 0.5 hour per day with no organic injury. The effects of MC were shown to be primarily anesthetic, with only a slight capacity to cause reversible injury to the lungs and liver. Based on this work, Torkelson et al. (1958) suggested that the maximum allowable concentration (ceiling) for MC be 500 ppm (2,700 mg/m^3) to avoid anesthetic effects.

In another study, Eben and Kimmerle (1974) exposed rats acutely (4 hours) and subchronically (8 hours/day, 5 times/week for 3 months) to 220 and 440 ppm (1,188 and 2,376 mg/m^3) and 200 ppm (1,080 mg/m^3), respectively. During the periods of exposure, the authors reported that "the animals did not differ in any way from their controls in behavior, appearance, or body weight gain. Hematologic examination performed at the end of the exposure period did not reveal any pathologic abnormalities". Liver and renal function tests and blood glucose were normal. At autopsy, the organs of the exposed rats were normal; organ weights did not deviate significantly from those of the control animals.

Prendergast et al. (1967) repeatedly exposed 15 Sprague-Dawley rats, 15 Hartley guinea pigs, 3 squirrel monkeys, 3 New Zealand albino rabbits, and 2

TABLE 5-4. ACUTE TOXICITY OF METHYL CHLOROFORM

Reference	Species	Route	Sex	LD_{50} mg/kg	95% confidence limits
Torkelson et al., 1958	rats	oral	M	14,300	12,100 to 17,000
Torkelson et al., 1958	rats	oral	F	11,000	9,500 to 13,000
Torkelson et al., 1958	mice	oral	F	9,700	
Torkelson et al., 1958	albino rabbits	oral	mixed	10,500	9,700 to 11,300
Torkelson et al., 1958	guinea pigs	oral	mixed	8,600	6,100 to 12,200
Takeuchi 1966	SM mice	i.p.	?	2,568	
Priestly and Plaa 1976	CF-1 Swiss mice	i.p.	M	4,008	3,558 to 4,522
Klaassen and Plaa 1966	Sprague-Dawley rats	i.p.	M	5,054	4,389 to 5,586
Torkelson et al., 1958	albino rabbits	dermal	mixed	15,800	
Adams et al., 1950	rats	inhalation	mixed	18,000[1]	
Plaa et al., 1958	Princeton mice	i.p.	M	16,000	12,700 to 21,400
Klaassen and Plaa 1967	Swiss-Webster mice	i.p.	M	5,080	4,140 to 6,010
Klaassen and Plaa 1967	dog	i.p.	M	4,140	
Gehring 1968	Swiss-Webster mice	i.p.	F	4,700	4,320 to 5,110
Woolverton and Balster	CD-1 mice	inhalation	M	22,240[2]	

[1] LC_{50} in ppm for 3 hr exposure; 7 hr exposure resulted in a LC_{50} of 14,250 ppm (12,950 to 15,675).

[2] LC_{50} in ppm for 30 min.

beagle dogs (8 hour/day, 5 days/week for 6 weeks) to 2200 ppm (11,880 mg/m^3) MC. The same number of animals were exposed continuously for 90 days to 135 ppm and 370 ppm (729 and 1,998 mg/m^3). The repeated exposure to 2200 ppm (11,880 mg/m^3) did not result in any deaths or visible signs of toxicity, although weight loss was observed in rabbits and dogs. The continuous exposure at 370 ppm (1,998 mg/m^3) did not cause any deaths, visible toxic signs, significant growth depression or biochemical, hematologic or pathologic changes. Continuous exposure to 135 ppm (729 mg/m^3) resulted in three deaths (2 rats and one rabbit) but no visible toxic signs or impaired growth in any of the survivors. Autopsy and subsequent histopathologic examination of the experimental animals revealed lung congestion and pneumonitis which may have been severe enough to have caused the deaths of the two rats and the rabbit, but which were not attributed to the exposure.

The acute behavioral and toxic effects of ethanol and inhaled MC alone and in combination have been examined by Woolverton and Balster (1981) on CD-1 albino mice. The animals were exposed to doses of MC ranging to 20,000 ppm (108,000 mg/m^3) for 30 minutes and then tested for behavioral decrements using the inverted screen test, a test that requires mice to climb to the top of an inverted screen. Lethality was measured at 24 hours. The inverted screen test was scored by counting the number of animals to fall off plus the number on the underside of the screen, expressing these numbers as a percentage. To test the effect of ethanol, animals were first intubated with varying doses of ethanol and then exposed to MC.

At the highest concentration of MC tested for behavioral effects (7,000 ppm; 37,800 mg/m^3), half of the affected mice recovered within 5 minutes after removal from the chamber and all recovered within 60 minutes. In contrast, at the highest dose of ethanol tested (4 g/kg), half of the animals recovered within 180 minutes and 80 percent recovered within 360 minutes. Twelve mice were used for each data point.

The EC_{50} for MC at 1 minute following a 30 minute exposure was in the range of 4,644 to 5,778 ppm (24,077 to 31,201 mg/m^3). The 24-hour LC_{50} was in the range of 22,080 to 22,404 ppm (119,232 to 120,981 mg/m^3). For ethanol, the ED_{50} was in the range of 1.7 to 2.7 g/kg and the 24-hour LD_{50} was in the range of 7.5 to 8.0 g/kg.

When the combined effect was evaluated, the investigators observed that low doses of ethanol and MC resulted in a greater-than-additive response both on behavioral and lethal endpoints; higher doses of ethanol were additive or less-than-additive with MC. In general, the LC_{50}/EC_{50} ratio of MC increased as the dose of ethanol increased (from 0 to 2.0 g/kg). One anomalous finding was at the ethanol dose level of 0.25 g/kg. In contrast to the potentiating effect of ethanol at other dose levels, this dosage increased the EC_{50} for MC, in comparison and reduced the LC_{50}/EC_{50} ratio for MC. This dosage of ethanol was not reported to have effects of its own on the parameters measured but apparently decreased the effects of MC. The combined effects data are shown in Table 5-5. Additional information is discussed in section 5.2.2.

TABLE 5-5. COMPARISON OF LETHAL AND BEHAVIORAL EFFECTS OF MC IN COMBINATION WITH ETHANOL

Ethanol dose (g/kg)	MC LC_{50} (ppm)	MC EC_{50} (ppm)	LC_{50}/EC_{50}
0	22,241	5,173	4.3
0.125	20,273	3,834	5.3
0.25	19,852	6,006	3.3
0.5	19,067	3,157	6.0
1.0	17,924	2,200	8.1
2.0	16,834	618	26.5

Source: Woolverton and Balster, 1981

Because the liver is a target organ for many chlorinated hydrocarbons, the effects of MC on hepatic function have been studied by a number of investigators.

McNutt et al. (1975) reported hepatic lesions in mice after continuous inhalation exposure to MC. CF-1 mice were exposed to determine the nature of pathologic alterations and to obtain data that would be useful in establishing acceptable levels for continuous exposures. Two levels were chosen: 1,000 ppm (5,400 mg/m^3) and 250 ppm (1,350 mg/m^3), the first in order to produce a definite, mild toxic effect and the other as an estimate of a concentration that might give a threshold response or possibly, no detectable effect. Mice were continuously exposed for up to 14 weeks with a weekly serial sacrifice. In the high exposure group (from 1 to 14 weeks of exposure), cytoplasmic alterations were observed in the centrilobular hepacytes upon electron microscopic evaluation. Alterations consisted of vesiculation of the rough endoplasmic reticulum with loss of attached polyribosomes and increased smooth endoplasmic reticulum, microbodies, and triglyceride droplets. Necrosis of individual hepatocytes occurred in 40 percent of the mice in the high exposure group, exposed for 12 weeks. The necrosis was associated with an acute inflammatory infiltrate and hypertrophy of Kupffer cells. Moderate liver triglyceride accumulation was evident and peaked at 40 mg/gm of tissue after 7 weeks of exposure; by 14 weeks the triglyceride level had decreased to 16 mg/gm.

In contrast, cytoplasmic alterations were described as mild to minimal in the 250 ppm (1,350 mg/m^3) group. Necrosis was not evident nor was fat accumulation elevated above control values. The authors concluded that the observed effects of MC were of a type similar to those produced by carbon tetrachloride but appeared to be much less severe.

As part of the same study described above, rats, dogs, and monkeys were also exposed continuously to 250 and 1,000 ppm (1,350 and 5,400 mg/m^3) MC. The most prominent observation in the rats was the presence of chronic respiratory disease which was found in 12 of 40 controls, 28 of 40 rats in the low exposure group and in 17 of 40 rats exposed to 1,000 ppm (5,400 mg/m^3). Other observations included focal areas of tubular dilation in the kidney. Since the incidence in the treated and control animals was similar, the observation was interpreted as being unrelated to the exposure. No evidence of fatty infiltration of the liver was observed in the treated animals. No adverse health responses were observed in dogs or monkeys that were related to exposure.

The combined effect of nominal concentrations of methylene chloride (100 ppm) and MC (1,000 ppm) in rats, mice, dogs, and monkeys was investigated by the Aerospace Medical Research Laboratory (1975). Animals were exposed continuously for 13 weeks. Though most animals were sacrificed, subgroups of each species were held for an additional period of time to determine the reversibility of any alterations that might occur. In rats, dogs, and monkeys, there were no significant differences from the control animals. In mice, however, there was a consistent finding in liver tissue of multifocal periacinal areas in which there was vacuolization of surrounding hepatocytes. Increased amounts of fat were observed in these periacinal areas. The effect was reversible and ameliorated within 14 days post-exposure.

Table 5-6 shows the relative hepatotoxic effect of MC in comparison to other chlorinated solvents. MC, according to Plaa et al., (1958) was judged to be the least hepatotoxic of the seven solvents investigated.

TABLE 5-6. THE RELATIVE HEPATOTOXIC EFFICACY OF CHLORINATED SOLVENTS

Compound	Relative hepatotoxic efficacy
1,1,1-trichloroethane	1
Tetrachloroethylene	3
Trichloroethylene	8
Sym-tetrachloroethane	12
1,1,2-trichloroethane	40
Chloroform	60
Carbon tetrachloride	190

Adapted from Plaa et al., (1958).

5.2.2 Nervous System and Behavior

There have been few animal studies of the effects of MC on the nervous system and behavior. In the animal studies that have been performed, effects of MC were either not observed or were observed at exposure levels of 4,000 ppm (21,600 mg/m^3) or greater (Table 5-7). The effects were upon motor abilities (Woolverton and Balster, 1981), vestibular control (Larsby et al., 1978) or on fixed-interval response rate (Moser and Balster, 1982). Studies apparently have not been performed upon such behaviors as learning or extinction, accuracy or efficiency of complex schedule-controlled behaviors or sensory discriminations. The endpoints that have been evaluated were studied in only a preliminary manner. Nervous system electrophysiology, neurochemistry, and neuropathology are other areas that warrant attention.

Exposure of 10 Sprague-Dawley male rats to 500 ppm (2,700 mg/m^3) was not found to significantly affect activity level. Rats were exposed by inhalation for 4 days, 6 hours/day. They were evaluated one and 17 hours after the last exposure in an open field setting. Ambulation, grooming, rearing, defecation, and urination were measured (Savolainen et al., 1977).

York et al. (1982) reported that exposure of female Long-Evans rats by inhalation before and/or during pregnancy to 2,100 ppm (11,340 mg/m^3) did not affect mating (anecdotal data) nor were open field activity, running wheel

TABLE 5-7. NERVOUS SYSTEM AND BEHAVIORAL EFFECTS OF MC IN LABORATORY ANIMALS

REFERENCE	SPECIES	SEX	EXPOSURE	EFFECTS
Savolainen et al. (1977)	Sprague-Dawley rats	Male adults	Inhalation 500 ppm, 4 days, 6 hour/day	No effect on activity level, amount of brain protein or RNA.
York et al. (1982)	Long-Evans rats	Females and offspring	Females exposed to 2100 ppm inhalation during and/or before pregnancy.	No effects on breeding in females or open field or running wheel activity in offspring.
Woolverton & Balster (1981)	CD-1 mice	Male adults	Inhalation of 3,500-7,000 ppm MC	EC_{50} for inverted screen climbing was about 5,200 ppm.
Moser & Balster (1982)	CD-1 mice	Male adults	Inhalation of 1,000-8,000 ppm MC	Reduced FI response rate beginning at 4,000 ppm.
Larsby et al. (1978)	Rabbit	?	Infusion of MC	Vestibular disturbances beginning at 75 ppm MC in arterial blood.

rates affected in the offspring. Open field tests were done on post-partum day 21 and running wheel tests from day 21 through day 110. Amphetamine administered to all groups before testing on days 110 through 120 did not alter running wheel rates with respect to controls.

Woolverton and Balster (1981) showed a concentration-related increase in the percent of CD-1 mice affected by MC inhalation on an inverted-screen climbing test. The EC_{50} for the 12 mice evaluated was about 5,200 ppm (28,080 mg/m^3). At a level of 7,000 ppm (37,800 mg/m^3), about 80 percent of the mice were affected. Tests were run beginning one minute after the termination of exposure. Ethanol doses, given prior to MC exposure, shifted the concentration-effects curve to the left by amounts depending upon MC concentration. It is quite possible that the observed decrements were due to vestibular rather than motor deficits.

The fixed interval response rate in adult male CD-1 mice was measured during inhalation exposure to 1,000, 2,000, 4,000, and 8,000 ppm (5,400, 110,800, 21,600, and 43,200 mg/m^3) MC (Moser and Balster, 1982). Response rate was reduced proportional to concentration in about 5 minutes after the initiation of exposure, beginning at 4,000 ppm (21,600 mg/m^3). It was unclear whether mice were trained in unexposed conditions.

An apparent disturbance in vestibular function due to MC was reported in rabbits by Larsby et al. (1978). MC was infused intravenously and the blood level of MC was monitored. At 75 ppm, rabbits began showing nystagmus during axial body tilts. A crude estimate made from the data reported by Astrand et al. (1973) indicated that 75 ppm MC in human arterial blood would result upon inhalation of air containing about 4,000 ppm (21,600 mg/m^3).

Protein and RNA content in the brains of Sprague-Dawley male rats at 0, 2, 3, 4, and 6 hours after the last exposure to 500 ppm (2,700 mg/m^3) MC for 4 days, 6 hours/day was measured by Savolainen et al. (1977). No effects were significant after Bonferroni correction for repeated t-tests (Benignus and Muller, 1982).

The available information indicates that, at high levels, MC acts as a CNS anesthetic and at somewhat lower levels it has CNS depressant effects. Until more pertinent information is available, very few conclusions about threshold effects are warranted.

5.2.3 Cardiovascular Effects

The cardiovascular effects produced by exposure to MC have been extensively studied in recent years. Halogenated alkanes, like many organic solvents, have been shown to sensitize the heart to catecholamines and at the same time produce cardiac depression.

Krantz et al. (1959) were the first to observe the cardiac depression produced by MC. Rats were deeply anesthetized with MC for 1 hour, at which time the hearts were immediately removed. Cardiac ventricular slices were promptly prepared and the oxygen uptake over the 1 hour period was measured. MC anesthesia was associated with a significant diminution (33.3 percent) in oxygen uptake of the myocardium. Blood pressure studies in dogs also revealed that anesthesia with MC elicited a depressed response. At the point of respiratory arrest, the blood pressure was reduced to approximately one-half its normal value. Ether anesthesia under similar conditions results in only a slightly depressed blood pressure. Six dogs and two rhesus monkeys were anesthetized and electrocardiograms recorded. The pattern of the electrocardiogram was essentially unaltered; the heart rate was increased and the T-wave was either flattened or inverted. At the point of respiratory arrest, a depressed S-T segment was observed; however, tachycardia was absent.

Herd and co-workers (1973, 1974) confirmed the initial observations of Krantz et al. (1959). They exposed dogs to 10,000 to 40,000 ppm (54,000 to 216,000 mg/m^3) MC per minute and observed a dose-dependent biphasic decline in arterial blood pressure. Since cardiac output increased initially, the initial decline in pressure (within 10 to 15 seconds after introduction of MC) was due to a decrease in total peripheral resistance (TPR). Injection of phenylephrine (pure alpha-agonist) reversed the peripheral vascular effects, indicating that MC does not act directly on the vascular musculature. The second phase of blood pressure decline was found to be associated with a decrease in myocardial contractility, reflected by a decline in both heart rate and stroke volume. Exogenous Ca^{++} reversed the MC-induced decline in myocardial contractility, but had no effect on the initial phase of peripheral vasodilation.

Taylor et al. (1976) exposed New Zealand white rabbits to a series of haloalkane chemicals. The animals were first anesthetized with sodium pentobarbital, then fitted with various cannulas allowing for the measurement of mean arterial pressure, left ventricular pressure, left ventricle dP/dt, cardiac output, stroke volume, heart rate, left ventricular end-diastolic pressure, central venous pressure, and peripheral vascular resistance. Table 5-8 lists the left ventricular and hemodynamic effects of 50,000 ppm (270,000 mg/m^3) MC. There was no effect on heart rate, left ventricular end-diastolic pressure, central venous pressure, or peripheral vascular resistance. During exposure, no cardiac arrhythmias were observed nor was there a significant change in pH, oxygen, or carbon dioxide tensions. While this level of exposure would probably be lethal in humans, it is many orders of magnitude greater than levels found in ambient air.

Numerous hydrocarbon compounds have been shown to sensitize the myocardium to catecholamines. In humans, for example, several unexplained deaths have been associated with solvent abuse or overexposure; ventricular fibrillation due to cardiac sensitization has been suggested as the underlying mechanism. The cardiac sensitization potential of MC has been investigated under various circumstances. In an early study, Rennick et al. (1949) reported MC and epinephrine induction of idioventricular rhythms in dogs. Attempts to induce MC anesthesia resulted in the sudden death, presumably of cardiac origin, of two animals. Ventricular extrasystoles and ventricular tachycardia were seen in five dogs under barbital anesthesia when epinephrine was injected after "repeated small doses" of MC. Maximum sensitization of the heart to epinephrine occurred after the administration of 0.25 to 0.4 ml/kg of MC. Further administration of this compound produced severe hypotension. Somani and Lum (1965), Lucchesi (1965), and Hermansen (1970) administered MC to dogs or mice as a method for induction of cardiac arrhythmias in studying and characterizing the sensitivity and specificity of adrenergic blocking agents.

Clark and Tinston (1973) evaluated the cardiac sensitizing potencies of 14 halogenated hydrocarbons in conscious beagle dogs. During the last 10 seconds of a 5 minute exposure, epinephrine (5 mg/kg) was injected intravenously (bolus). As a control, epinephrine was given prior to exposure and also 10 minute after the end of exposure. The electrocardiogram (lead II) was monitored continuously during this procedure. Several dose levels of the chemical being tested were used, each differing by a factor of two. The concentration at which 50% (EC_{50}) of the animals could be sensitized was calculated by a moving average interpolation. The mean concentration of MC which produced cardiac sensitization in 50% of the animals tested was 7,500 ppm (40,500 mg/m^3). The authors found that they could directly predict the EC_{50} for cardiac sensitization by knowing the vapor pressure at 37°C and the

TABLE 5-8. LEFT VENTRICULAR AND HEMODYNAMIC EFFECTS OF METHYL CHLOROFORM

	Mean Arterial Pressure (mmHg)	Left Ventricular Pressure (mmHg)	Left Ventricle dP/dt (mmHg/sec)	Cardiac Output (ml/min)	Stroke Volume (ml)
Pre-exposure	69±5	97±3	3792±325	423±31	1.47±0.09
1 minute post exposure[a]	45±4	70±5	1433±163	266±26	0.92±0.07

[a] All values at 1 minute exposure are significantly different from pre-exposure values at $p < 0.05$.

Source: Taylor et al. (1976).

partial pressure at the EC_{50}. Table 5-9 lists the EC_{50} for a number of different halogenated hydrocarbons. MC appears to be a highly potent sensitizing agent at near anesthetic levels, with tetrachlorodifluoromethane being the most potent in the series. The authors concluded that cardiac sensitization was probably a structurally nonspecific action and that it was physically toxic.

TABLE 5-9. CONCENTRATION OF CHEMICALS CAUSING CARDIAC SENSITIZATION AND THEIR PHYSICAL PROPERTIES (IN PPM)

Chemical	EC_{50}	Vapor pressure at 37°C (mmHg) P_s	Partial pressure at EC_{50}(mmHg) P_{cs}	Relative saturation for cardiac sensitization P_{cs}/P_s
Tetrachlorodifluoromethane	1,200	99	2	0.02
Carbon tetrachloride	5,000	190	4	0.02
Trichloroethane (MC)	7,500	210	6	0.03
Halothane	20,000	480	15	0.03
Trichlorotrifluoroethane	10,000	524	8	0.02
Methylene chloride	24,000	661	18	0.03
Trichlorofluoromethane	12,500	1,186	10	0.01
Dichlorofluoromethane	25,000	2,052	19	0.01
Dichlorotetrafluoroethane	100,000	2,310	76	0.03
Vinyl chloride	50,000	4,218	38	0.01
Propane	200,000	9,538	153	0.02
Bromotrifluoromethane	200,000	15,276	153	0.01
Chlorotrifluoromethane	800,000	40,698	610	0.02

Source: Clark and Tinston (1973).

Reinhardt et al. (1973) investigated five commonly used industrial and household solvents, including MC, in order to rank their relative cardiac-sensitization potentials. In this screening test, the investigators conducted experiments on unanesthetized, healthy male beagle dogs that had been trained to breathe through a one-way face mask while supported in a standing position by a sling. The dog inhaled house air for 7 minutes and the test compound for 10 minutes. Epinephrine (8 µg/kg in 1 ml of normal saline) was injected i.v. in 9 seconds as a control dose after 2 minutes of breathing air, and as a challenge dose after 5 minutes of breathing the test compound. The 10-minute interval between the two doses was found to be adequate to prevent additive effects when tested in 13 control dogs subjected to the same procedures, but with air substituted for the test compound. A positive response was considered to be either cessation of cardiac output (ventricular fibrillation) or the development of an arrhythmia that was not observed following the control dose and that was considered to pose a serious threat to life (multiple, consecutive beats of ventricular origin). The concentration of the test compound being delivered to the animal was determined by gas chromatography at 2-minute intervals.

The results obtained for MC indicated no response in 12 dogs at a nominal concentration of 0.25 percent (2,500 ppm) (V/V) and a marked response in 3 of 18 dogs exposed at a nominal concentration of 0.5 percent (5,000 ppm) (V/V); 12 of 12 responded at a nominal concentration of 1.0 percent (10,000 ppm) (V/V). However, unlike the other substances, MC-induced ventricular fibrillation in the animals from the 1.0 percent group reverted to multiple consecutive ventricular beats within a matter of seconds and eventually recovered to a normal cardiac rhythm. The results confirm earlier reports that MC is capable of sensitizing the dog heart to exogenous epinephrine although none of the dogs died.

Trochimowicz and co-workers (1976) were concerned with the problem of haloalkane-induced cardiac sensitization in patients who had survived myocardial infarction. They induced myocardial infarction in beagle dogs and tested for cardiac sensitization to epinephrine after exposure to MC at levels of 2500, 3700, and 5000 ppm (13,500; 19,980; and 27,000 mg/m^3). Myocardial infarction did not significantly alter the threshold for cardiac sensitization. There was no greater potential for cardiac sensitization among dogs having recovered from myocardial infarction compared to normal healthy animals.

Rabbits have recently been shown, by Carlson (1981), to respond to a combined inhalation exposure to MC and a challenge dose of epinephrine, resulting in spontaneous arrhythmias (premature ventricular contractions). Controls did not exhibit arrhythmias at an exposure level of 5,600 ppm (30,240 mg/m^3) up to 60 minutes, in the absence of a challenge dose of epinephrine. Induction of arrhythmia was examined also by first administering phenobarbital (40 mg/kg) i.p. daily for 4 days prior to exposure or administering microsomal mixed function oxidase inhibitors. Although none of the results reported by Carlson (1981) were statistically significant, both inhibitors appeared to increase the responsiveness of the heart to arrhythmias when compared to controls. Fifty percent of the rabbits administered SKF-525A (50 mg/kg) 30 minutes prior to exposure exhibited arrhythmias after 7.5 minutes of exposure to MC, following a challenge dose of 2 ug/kg epinephrine. In both inhibitor groups there was a rapid return to normal cardiac rhythm during the recovery period.

Blood analysis revealed trichloroethanol (TCE) as the principal metabolic product. Phenobarbital resulted in an increase in blood TCE but it was not statistically significant. The inhibitors resulted in an increased amount of blood MC. This observation and the tendency of the inhibitor-treated animals to show a higher incidence of arrhythmias led the investigators to conclude that MC, not the metabolites, was responsible for the arrhythmias.

At sufficiently high levels (in the thousands of ppm, for durations of exposure on the order of minutes), MC has been reported to cause significant alterations in cardiovascular function in a variety of experimental animals, including mice, rabbits, dogs, and possibly monkeys. The concentration levels reported for these effects are generally more than an order of magnitude greater than the current TWA for humans of 350 ppm (1,890 mg/m^3) and are likely to be lethal to the experimental animals if the exposure is continued for more than several minutes. Indeed, some experimenters appeared to find it necessary to increase the oxygen content of the inspired gas/air mixture to 40 percent in order to prevent asphyxia of the exposed animals. That they appeared unable to find effects at lower exposure levels is noteworthy. Also noteworthy is the fact that many of the investigators reported complete recovery of the animals if the exposure was stopped. This was the case, for example, with the dog experiments of Reinhardt et al. (1973) and Trochimowicz et al. (1976). At necropsy following sacrifice of several of the animals several months after exposure, there was no pathology attributable to the exposure *per se*. Delayed cardiotoxicity from acute exposure has not been demonstrated.

5.2.4 Dermal Effects

MC was one of seven solvents tested for dermal effects by Kronevi et al. (1981). Guinea pigs (17) were anesthetized with pentobarbital, i.p., and one ml of an inhibited formulation of MC was applied to an area (3.1 cm^2) on the clipped back skin, through a hole in the cover glass. The hole was closed with a small piece of glass glued with α-cyanoacrylate. At 15 minutes, 1.4, and 16 hours, glass rings were removed and whole skin specimens from exposed sites were excised and fixed in 10% formalin. Samples from adjacent non-exposed sites were taken as controls.

Pyknotic nuclei were seen in all layers of epidermis, beginning with the 15 minute sample. It was reported that degeneration of nuclei progressed in proportion to increased exposure times involving pyknosis and karyolsis. A marked intercellular edema was observed after 15 minutes; edema was hardly recognizable at 1 hour and not at all after 4 and 16 hours; at this time, it was totally replaced by junctional separation. A slight, diffuse pseudo-eosinophilic infiltration appeared in the upper part of the dermis after 4 hours and became severe after 16 hours.

Karyopyknosis was a finding common to all the solvents evaluated. Karyolysis, intercellular edema and junctional separation was common to most of the solvents.

The results of this one study suggest that skin contact with MC be minimized.

5.3 TERATOGENICITY, MUTAGENICITY, AND CARCINOGENICITY

It has been proposed that the susceptibility of individuals to cancer is related to their ability to metabolize carcinogenic compounds to benign molecules and, conversely, to their capacity to metabolize benign compounds to carcinogens (Kellerman et al., 1973). Susceptibility may be partly explained by genetic differences involving enzymatic pathways, repair mechanisms, and immune response mechanisms. An assessment of the carcinogenic potential of MC must therefore take into account tissue concentrations of MC during typical exposures as well as the extent and nature of MC metabolism. Both these aspects have been extensively reviewed in Chapter 4. It can be concluded that MC is metabolized in humans to a very small extent; about 6 percent or less of the body dose is converted to carbon dioxide, trichloroethanol, and trichloroacetic acid. The mechanism of this overall reaction is unknown. The available studies on carcinogenicity and teratogenicity are summarized in Table 5-10.

5.3.1 Teratogenicity, Embryotoxicity, and Reproductive Effects

5.3.1.1 Overview--The basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" were summarized by The Office of Pesticides and Toxic Substances (U.S. EPA, 1980c) as follows:

> Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring *in utero*. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis edema, and increased incidence of supernumary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.
>
> In view of the spectrum of effects at issue, the Agency suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to *in utero* exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.
>
> Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up". Unless a

TABLE 5-10. ONCOGENIC AND TERATOGENIC TESTING OF METHYL CHLOROFORM

Test System	Species	Reference	Results
Teratogenicity			
Organogenesis	Chick embryo	Elovaara et al., 1979	Positive; high toxic dose, skeletal abnormalities
Mammalian gestation	ICR Swiss mice (drinking water)	Lane et al., 1982	Negative
Mammalian gestation	Sprague-Dawley rat, Swiss-Webster mice (inhalation)	Schwetz et al., 1975	Negative
Mammalian gestation	Long-Evans rat (inhalation)	York et al., 1982	No teratogenic effects but slight reversible delay in development
Carcinogenicity			
NCI bioassay - 2 yr	Osborne-mendel rats, and $B_6C_3F_1$ mice and oral gavage; 750 and 1500 mg/kg (rat)	NCI, 1977; NCI Clearinghouse on Environmental Carcinogens, 1977	Inconclusive; high animal mortality
Industry bioassay - 1.5 yr	Sprague-Dawley rat, inhalation 875 and 1750 ppm		Negative

series of specific kidney function tests are performed on the neonate, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting after effects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/fetal effect is one species might, under a given agent, react in another species in a more serious and irreversible manner.

It is not possible, on the basis of limited available data, to define the full potential of MC to produce adverse teratogenic or reproductive effects. Human epidemiology studies are difficult to conduct in order to evaluate the effects of MC on the exposed population. Each of the available mammalian studies had methodological drawbacks that do not allow for conclusive evaluation of the ability of MC to produce a teratogenic response over a wide range of doses, which should include doses high enough to produce signs of maternal toxicity and lower doses which do not produce this effect. Some of the teratology studies used rats and mice and only single doses of MC which produced signs of maternal toxicity. Other studies in chicken embryos have indicated that MC disrupts embryogenesis in a dose-related manner (Elovaara et al., 1979). However, since administration of MC directly into the air space of chicken embryo is not comparable to administration of a dose to animals with a placenta, it is not possible to interpret this result in relationship to the potential of MC to cause adverse human reproductive effects.

5.3.1.2 Human Studies--No clinical reports associate maternal exposure to methyl chloroform with congenital malformations in offspring. No epidemiological studies have been performed.

5.3.1.3 Animal Studies--All studies performed to date in mammals have been done in rats and mice. On the basis of these studies, it does not appear that short- or long-term exposure to MC results in teratogenic effects in rats or mice. Delays in fetal development have been observed in both species, but these are believed to be reversible effects. The inhalation studies individually evaluated dosages twice the maximum excursion limit for short-term exposures of rats and mice and six times the maximum excursion limit for long-term exposures of rats. In addition, no multigenerational studies of mammalian reproductive performance have been performed under conditions of inhalation exposure.

5.3.1.3.1 Rats--Schwetz et al. (1975) report results from Sprague-Dawley rats exposed via inhalation to 875 ppm (4,725 mg/m^3) of MC for 7 hours daily on days 6 through 15 of gestation (Day 0 = the day sperm were observed in smears of vaginal contents). Control rats were exposed to filtered air. Dams were evaluated for body weight gain, food consumption and various organ weights. Maternal carboxyhemoglobin level determinations were performed on blood samples collected via orbital sinus puncture immediately following the third and tenth (last) exposure. One-half of the fetuses in each litter were examined for soft-tissue malformations (free-hand sectioning), and one-half were stained and examined for skeletal malformations. One fetus in each litter was randomly selected and evaluated using histological techniques following serial sectioning.

Twenty-three litters from dams exposed to 875 ppm (4,725 mg/m^3) of methyl chloroform were evaluated. No effect was observed on maternal body weight or food consumption. The mean absolute liver weight was increased as compared with control, however the mean relative liver weight was unchanged. No embryotoxic or teratogenic effects were observed which were attributable to maternal MC exposure.

York et al. (1982) exposed female Long-Evans rats by inhalation to dosages of 2100 ± 200 ppm (11,340 ± 1,080 mg/m^3) MC for 6 hours daily, 5 days per week, in the following regime: (1) two weeks prior to mating through day 20 of gestation; (2) two weeks prior to mating only; (3) throughout gestation only; (4) controls which were exposed to filtered air before and during pregnancy. Day 1 of pregnancy was designated as the day spermatozoa were observed in smears of vaginal contents. One-half of each group was sacrificed on day 21 and assessed for signs of maternal toxicity, embryotoxicity or teratogenicity. The other one-half were allowed to deliver young naturally and the young were later evaluated for behavioral alterations and for observation of gross lesions. When possible, litters were culled to four pups of each sex on day 4 postparturition and to two pups of each sex at 21 days postpartum. Pups were evaluated for behavioral effects (see 5.2.2) and carcinogenicity. Surviving rats were sacrificed and necropsied at 12 months of age.

York et al. (1982) reported no teratogenic effects due to exposure but both total fetal body weight and male fetal body weight were significantly depressed in litters when dams were exposed during pregnancy. Delayed ossification was more frequent in fetuses which had been exposed before and during pregnancy, and a delay in the development of the kidney was a more frequent observation in fetuses in the group exposed prior and during gestation.

These effects are thought to be indicative of a slight delay in development since they occurred in more than 5 percent of the population, including controls as well as exposed. No treatment-related behavioral effects were observed in the pups, nor were significant signs of maternal toxicity observed.

5.3.1.3.2 Mice--Schwetz et al. (1975) exposed Swiss-Webster mice to 875 ppm (4,725 mg/m^3) of MC, twice the maximum excursion limit, for 7 hours daily, on days 6 through 15 of gestation (day 0 = day a vaginal plug was observed). Dams were Caesarean sectioned on day 18 of gestation and thirteen litters were evaluated. Methodology similar to that described previously (Schwetz et al. 1975) in rats was used, with the exception that food consumption was not monitored.

Mean and relative maternal liver weights in the exposed mice were slightly but not statistically decreased. Also, fetuses from the exposed group were slightly but not statistically smaller (both the crown-rump length and the body weight). This type of observation is thought to indicate a slight, but perhaps reversible delay in fetal development. No increased soft tissue or skeletal variations of the fetuses were attributable to maternal exposure to MC.

Lane et al. (1982) investigated the effects of MC on the reproductive capability of ICR Swiss mice using a multigenerational reproductive protocol modified to include screening for teratogenic and dominant lethal effects. Animals were administered MC in drinking water for 35 days, then 10 male and 30 female mice (parental generation F/0) were mated to produce the first set of offspring (FIA). After the F/1A were weaned, the F/0 adults were remated to produce the second set of offspring (F/1B). A parental stock was chosen from the F/1B litter (30 females, 10 males) to produce a second generation of offspring (F/2A). After weaning the F/2A, the F/1B adults were remated to produce offspring (F/2B) for use in teratology and dominant lethal screening tests.

The MC (Aldrich Chemical Co. 95% pure with 3% dioxane) was dissolved in a solution of Emulphor EL-620 (GAF Corp. Kinden, New Jersey) (1% Emulphor dissolved in deionized water) then further diluted with deionized water to concentrations of 0.58, 1.75, and 5.83 mg/ml or a nominal dose of approximately 100, 300, and 1000 mg/kg/day (assuming a 35 g mouse consumes 6 ml/day). The test animals were continuously maintained on MC solutions or control solution (deionized water, or water containing 0.17 mg/ml p-dioxane dissolved in 1.0%

Emulphor solution). Fresh drinking solutions were prepared twice weekly and placed in amber glass bottles with cork stoppers and stainless steel drinking tubes. The authors reported no decreases in the amount of fluid consumed in either MC or the solvent control groups.

In this study MC produced no treatment related signs of toxicity such as lowered body weights or gross pathological changes in major organs. There were no significant differences in the fertility index or gestation index observed in the F/1A, F/1B, or F/2A generations. There were sporadic incidences of increased mortality throughout the generations but these were not dose-related. There was no difference in the litter sizes at birth, pup body weights, survival of pups at days 4 and 21 of birth. There was a decrease in the survival indices for the F/2A generation as compared to values for the F/1A and F/B generation but these decreases were not dose related. In addition, in the dominant lethal screening there were statistically significant differences; however, both increases as well as decreases were observed in the ratio of dead to live fetuses. In the teratology screening, the continuous administration of MC produced no apparent adverse reproductive effects, nor visceral or skeletal abnormalities. In the lowest dosage groups, none of the females had copulating plugs which made it impossible to time the pregnancies; therefore, no teratological examinations were performed. The reason for this effect was not disclosed in the paper.

In conclusion, this study is inadequate from the standpoint of determining whether MC has the potential to cause adverse reproductive or teratogenic effects because animals were not given doses high enough to produce overt signs of toxicity. Another confounding factor is that a determination of the amount of MC that may have partitioned into the head space of the inverted drinking solution bottles was not made (Lane, personal communication, 1982). Thus, it is not clear how much MC each animal received between solution changes. It had been determined, however, that MC was stable in solution, based upon measurements of stored drinking solutions. It appears that under the conditions of the experiment, mice (Swiss ICR) administered MC in drinking water (nominal doses of 100, 300, and 1000 mg/kg/day) did not exhibit any observable adverse reproductive effects.

5.3.2 Mutagenicity

Methyl chloroform (MC) has been tested for its ability to cause gene mutations in bacteria, Drosophila, and yeast; chromosome aberrations in rats;

micronuclei in mice; and unscheduled DNA synthesis in mouse and rat liver cells in vitro. Studies have also been reported on the ability of MC to reach germinal tissue and cause adverse effects there. These studies are evaluated and discussed below.

5.3.2.1 Gene Mutations in Bacteria--Several reports have been prepared about the mutagenicity of MC in bacteria; all were conducted using the Salmonella/mammalian microsome system (Table 5-11).

Because of MC's volatility and low solubility in water, special precautions should be taken to ensure adequate exposure of test organisms. No such precautions were taken in the tests conducted by Litton Bionetics (1975) for Dow Chemical Company. In this study various formulations of MC (i.e. 99+%, 96%, 95.65%, and 93.75%) were assayed for mutagenicity in Salmonella strains TA1535, TA1537, and TA1538, both with and without metabolic activation. A number of separate experiments (both liquid suspension tests and spot tests) were conducted each with and without an S9 mix prepared from various tissues (liver, lung, testes) of PCB-induced mice, rats, or monkeys. No information was provided concerning the stabilizers or other components present in the formulated samples tested. Preliminary toxicity testing was conducted in association with the liquid suspension assays to determine the appropriate test doses; the test doses used were different for each formulation and are presented in Table 5-11. The contractor reported that the formulated samples were not soluble in the aqueous testing environment and stated, for the 99+% formulation, that "the toxicity from test to test was quite variable depending upon the ability to effectively disperse the compound in the testing medium."

The low solubility of MC coupled with its high volatility raise the concern that exposure of the test organisms may have been minimal in the tests conducted by Litton Bionetics. Salmonella tester strain TA1535 was reported to exhibit a reproducible mutagenic response to the 99+% formulation in suspension tests, but the response was considered by Litton Bionetics as equivocal for the following reasons:

> A. "The positive response is only evident at high dose levels which generally result in low population survivals (high toxicity). Thus, one cannot exclude some type of selection.
>
> B. The data from activation plate tests does not indicate any activity."

TABLE 5-11. MUTAGENICITY TESTING OF METHYL CHLOROFORM: GENE MUTATIONS IN BACTERIA

Reference	Test System	Strain	Activation System	Concentration		Result	Comment
				Formulation	%		
Litton 1975	*Salmonella*/S9 spot test and liquid suspension test	TA1535 TA1537 TA1538	S9 prepared from PCB-induced liver, lung and testes from adult male animals (ICR random bred mice, Sprague-Dawley rats, and *Macaca mulatta* monkeys.)	99+ 96 95.65 93.75	5.0 2.5 2.0 1.0 1.0 0.5 0.5 0.25	99+% formulation reported positive with TA1535 both with and without metabolic activation. Result was reported to be repeatable. The other formulations were reported to be negative.	1. All formulations reported to be not soluble at test concentrations. 2. Toxicity reported to be variable. (In half of the tests, as many or more survivors were observed at the high dose compared to the low dose). 3. Strains TA98 and TA100 were not available at the time the test was conducted. 4. No information about the identity of the stabilizers and other components. 5. No special precaution taken to prevent evaporation of the compound, but test reported to be conducted in liquid suspension.
Gocke et al. 1981	*Salmonella*/S9 conducted in sealed desiccators	TA1535 TA1537 TA1538 TA98 TA100	PCB-induced rat liver S9 mix	ml/desiccator 0.5 1.0 1.5 2.0		Dose-related fourfold increase in TA1535.	1. Purity not given but reported to have correct elementary analysis and melting point.
Miyata et al. 1981	*Salmonella*/S9 preincubation assay	TA1535 TA1537 TA92 TA94 TA98 TA100	PCB-induced rat liver S9 mix	µl/plate 30 100 300 1000 3000		Negative. Toxicity observed at two highest doses	1. Protocol precludes comparison with studies employing airtight chambers.

TABLE 5-11. (Continued)

Reference	Test System	Strain	Activation System	Concentration	Result		Comment
					Sample (without activation)		
Snow et al. 1979	Salmonella/S9 conducted in sealed chambers	TA100	Methyl chloroform-induced Syrian golden hamster liver S9 mix.	Dose*	Aldrich	PPG	1. Only one strain tested. 2. Results indicate ability of stabilized MC to mutate bacteria with or without metabolic activation when precautions are taken to ensure exposure of test organisms. The response is somewhat greater with S9 mix. 3. Linear dose-responses obtained with no significant differences found between Aldrich or PPG samples at any concentration tested. 4. Protocol not completely described. 5. Purity of Aldrich sample not reported. Purity of the PPG sample reported to be high but information about composition not provided.
				0	130 ± 15†	136 ± 19	
				500	240 ± 10	230 ± 4	
				750	272 ± 2	275 ± 22	
				1000	298 ± 25	286 ± 20	
				1500	344 ± 10	365 ± 31	
					(with activation)		
				0	128 ± 13	128 ± 13	
				500	306 ± 22	285 ± 16	
				750	354 ± 10	347 ± 13	
				1000	393 ± 9	384 ± 20	
				1500	449 ± 31	456 ± 23	
Nestmann et al. 1980	Salmonella/S9 conducted in sealed desiccators	TA98 TA100 TA1535	PCB-induced rat liver S9 mix	ml/desiccator 0.1 0.5 1.0	Mutagenic in TA100 (up to 2.5-fold increase) and TA1535 (up to 6-fold increase).		1. Purity of test sample unknown. 2. Experimental values not given.

*µl/5.6-liter Billups-Rothenberg Modular Incubator Chamber.

†values represent mean ± SD of revertant counts from three plates/dose.

TABLE 5-11. (Continued)

Reference	Test System	Strain	Activation System	Concentration	Result			Comment
				MC(ppm)	Butylene Oxide (ppm)	No Activation	Activation	
Domoradzki 1980	Salmonella/S9 conducted in sealed dessicators		PCB-induced rat liver S9 mix					1. Unpublished results from tests with TA1535. 2. Positive and negative controls run concurrently for each experiment, but the use of nonvolatile positive controls is not appropriate experimental design. 3. Spontaneous controls are given as a range of values; the highest value (i.e., 97) is too high for TA 1535. 4. Actual concentrations achieved in exposure chambers and agar not determined. 5. Data consistent with hypothesis that mutagenic activity in bacteria is due to stabilizers but do not support it unequivocally.
	Methyl chloroform containing 380 ppm butylene oxide	TA1535		330,621	126†	Toxic	Toxic	
				165,310	63	Toxic	Toxic	
				82,655	31	Toxic	Toxic	
				41,328	16	21 ± 7	11 ± 4	
				41,328	16	23 ± 7	17 ± 2	
				0	0	14 - 24	23 - 97	
	CHLOROTHENE VG Solvent*			165,310	1207	Toxic	Toxic	
				41,328	302	98 ± 7	119 ± 9	
				41,328	302	117 ± 25	131 ± 9	
				20,664	151	47 ± 12	45 ± 4	
				20,664	151	59 ± 6	70 ± 8	
				0	0	14 - 24	23 - 97	
	1,2-Butylene oxide				1198	362 ± 30	419 ± 29	
					599	218 ± 9	217 ± 12	
					298	91 ± 15	97 ± 7	
					298	89 ± 20	133 ± 4	
					149	64 ± 4	66 ± 4	
					149	40 ± 2	52 ± 2	
					74	39 ± 5	33 ± 7	
					39	34 ± 7	38 ± 3	
					0	12 - 16	26 - 43	

*Trademark of the Dow Chemical Company.

†Amount calculated to be present in corresponding amount of MC.

TABLE 5-11. (Continued)

Reference	Test System	Strain	Activation System	Concentration	Result				Comment
				%	No Activation		Activation		
Williams and Shimada 1983	Salmonella/S9 conducted in gastight exposure chambers		PCB-induced rat liver S9 mix						1. Unpublished results from tests with TA1535. 2. Cytotoxicity tests performed on small populations of cells may not reflect cytotoxicity to large populations of cells on experimental plates. 3. Data are consistent with the hypothesis that the mutagenic activity is due to stabilizers, but do not demonstrate it absolutely.
	Methyl chloroform (stabilized)	TA1535		10	45 ± 10*(1.3)		44 ± 11 (2.2)		
				7.5	267 ± 49 (7.4)		258 ± 57 (4.8)		
				5.0	358 ± 31 (89)		358 ± 94 (68)		
				2.5	268 ± 22 (100)		222 ± 33 (95)		
				1.0	32 ± 7 (81)		22 ± 11 (97)		
	Methyl chloroform (low stabilized)			10	93 ± 24 (4.5)		122 ± 32 (1.3)		
				7.5	25 ± 7 (4.1)		26 ± 9 (3.7)		
				5.0	32 ± 7 (69)		23 ± 8 (70)		
				2.5	26 ± 9 (90)		25 ± 7 (94)		
	Vinyl chloride			7.5	221 ± 10 (100)		979 ± 74 (78)		
	Spontaneous revertants			0	18 ± 8		23 ± 5		
							TA100 Revertant Colonies		
					Source	Dose	+S9	-S9	
Simmon et al. 1977	Salmonella/S9 conducted in sealed desiccator	TA1535 TA1537 TA1538 TA98 TA100	PCB-induced rat liver S9 mix.	0, 100, 200, 300, 400, 500, 750, 1000 µl/9-liter desiccator (Extrapolated from figure 21 and personal communication with Drs. Mortelmans and Wang).	Aldrich	0	124 ± 11†	129 ± 12	1. Results indicate ability of commercial samples of MC to mutate bacteria with and without metabolic activation when precautions are taken to ensure exposure of test organisms. 2. Data were presented for TA100 only. 2. Data were presented for TA100 only.
						100	130 ± 17	146 ± 14	
						200	127 ± 18	181 ± 6	
						300	174 ± 20	191 ± 24	
						400	148 ± 13	135 ± 12	
						500	210 ± 4	221 ± 9	
						750	264 ± 19	218 ± 24	
						1000	283 ± 8	239 ± 4	
					MCB (Crude)	0	133 ± 4	118 ± 6	
						100	126 ± 14	131 ± 6	
						500	159 ± 16	143 ± 4	
						750	157 ± 26	158 ± 14	
						1000	202 ± 5	175 ± 7	
						1500	226 ± 21	206 ± 27	
					MCB (Purified)	0	169 ± 9	172 ± 16	
						100	184 ± 16	177 ± 15	
						500	224 ± 20	196 ± 15	
						750	247 ± 14	203 ± 9	
						1000	201 ± 8	201 ± 12	
						1500	239 ± 7	251 ± 32	
						2000	310 ± 44	258 ± 21	

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*Values represent mean ± standard deviation of revertant counts from 3 to 6 plates. Values in parentheses represent percent of surviving cells as estimated from cytotoxicity test.

†Values represent mean ± SD of revertant counts from at least three plates.

The data presented in the report are contrary to the conclusions. Cell survival in the high dose group was in fact equal to or greater than that in the low dose group in two out of four experiments, including the experiment in which the highest mutation frequency was observed. Thus, there is little basis for believing that cytotoxicity resulted in a spurious positive result. Futhermore, the negative results from the plate tests with activation do not provide convincing evidence of nonmutagenicity, because the plate tests performed in this experiment were spot tests. The proper performance of bacterial spot tests requires that the compound be soluble in water, so that it diffuses through the agar from the point of application forming a concentration gradient. Because MC is volatile and was not soluble under the conditions of these tests, the spot tests are considered to be inadequate.

Miyata et al. (1981) tested MC (source and purity not given) for mutagenicity using a liquid preincubation assay. Bacteria (i.e., TA1535, TA100, TA1537, TA98, and TA94) were exposed to MC in liquid suspension for 20 minutes before plating. The protocol was designed to prevent evaporation and ensure exposure of the bacteria; however, because of the relatively short preincubation period and the physical properties of MC, actual exposure may have been minimal. The testing was conducted both with and without PCB-induced rat liver S9 mix for metabolic activation. Five concentrations of MC, ranging from 30 to 3000 µg/plate, were employed. A negative response was obtained (see Table 5-11); toxicity was observed at the two highest dose levels, but no increase in revertant counts was observed.

In reports by Henschler et al. (1977) and by Taylor (1977), MC was reported to be negative. However, these reports cannot be accepted as valid because the report by Henschler et al. presents no data, and the unpublished report by Taylor lacks data from concurrent positive controls.

Several tests of the mutagenic potential of commercial samples of MC have been conducted in bacteria using protocols that allow gaseous exposure without excessive evaporation. All such tests that ensure exposure of the indicator organisms have given positive results in strains TA100 and/or TA1535 (Simmon et al. 1977, Snow et al. 1979, Nestmann et al. 1980, Domoradzki 1980, Gocke et al. 1981, and Williams and Shimada 1983). It is possible that this activity is due to mutagenic stabilizers. For instance, Henschler et al. (1980) analyzed 22 commercially available samples of MC by gas chromatography/mass spectrometry and identified substances which have been reported to be mutagenic in most of the samples. The substances identified included vinylidene chloride, 1,1-dichloroethane, trichloroethylene, 1,1,2-trichloroethane, 1,2-epoxybutane, and others. Experiments are needed to resolve whether the mutagenicity of commercial MC is due to MC per se or to stabilizer components. Perhaps fractionation of a mutagenic commercial sample and subsequent demonstration that the activity is due to substances in peaks other than the one containing MC would resolve this issue.

The tests performed by Simmon and co-workers (1977) were conducted using the standard battery of Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, both with and without PCB-induced rat liver S9 mix for metabolic activation. The concentrations used for testing were 0, 100, 200, 300, 400, 500, 750, and 1000 μl/9-liter desiccator. A weak dose-related response was observed for TA100 both with and without metabolic activation, showing that commercially available MC (from Aldrich Chemical Company) possesses mutagenic activity in Salmonella. When the study was repeated with the same sample of MC, no significant mutagenicity was observed (Dr. Yi Wang, SRI International, personal communication, 1982). Subsequently, a sample from Matheson, Coleman, and Bell (MCB) was tested by Simmon and colleagues, and weakly positive dose-related increases in mutant frequency were obtained at doses ranging from 100 to 500 μl/dl desiccator. This sample was analyzed (Dr. Ronald Spanggord, SRI International, personal communication, 1982) and found to be 84% pure and to contain several contaminants, including p-dioxane; 1,1-dichloroethylene; nitromethane; 1,1-dichloroethane; chloroform, and the reported mutagens trichloroethylene and 1,2-epoxybutane. After distillation to increase the purity to 98% and acid hydrolysis to remove the 1,2-epoxybutane contaminant, the material was reanalyzed and tested for mutagenicity. The MCB samples showed nearly the same mutagenic responses before and after purification (Dr. Kristien Mortelmans and Dr. Yi Wang, SRI International, personal communication, 1982). These results are consistent with the hypothesis that the response is due to MC itself and not to mutagenic stabilizers or contaminants. However, unidentified impurities (1-2% of the sample) remained in the purified MCB sample; thus, it is not possible to ascribe the mutagenic activity of this sample to MC unequivocally.

In the studies of Snow et al. (1979), two samples of MC were tested in Salmonella strain TA100 both with and without an S9 metabolic activation mixture prepared from the livers of MC-induced Syrian golden hamsters. Precautions were taken to prevent evaporation by exposing the bacteria to doses of

0, 500, 750, 1000, and 1500 µl in 5.6-liter Billups-Rothenberg modular incubator chambers. Similar linear dose-responses were observed (see Table 5-11) for each of two MC samples. One of the samples was from Aldrich Chemical Company (97% MC stabilized with 3% p-dioxane) and the other was from PPG Industries (also estimated to be 97% pure, but the stabilizers were not reported). These samples were analyzed by mass spectrometry three years after the testing was conducted (Dr. Stephen Nesnow, U.S. EPA, personal communication). The Aldrich sample was found to contain dioxane; 1,1- or 1,2-dichloroethylene; nitromethane; trichloroethylene; methylisobutyl ketone; 1,1,2-trichloroethane; and toluene. Acetone; nitromethane; methyl ethylketone, an unidentified substance tentatively identified as dimethyl formamide; toluene; trichloroethylene, and 1,1- or 1,2-dichloroethylene were found as contaminants in the PPG sample. Of these impurities, only 1,2-dichloroethylene (vinylidene chloride) and trichloroethylene have been reported in the literature to be mutagenic. It is not known whether these chemical compositions differ from those of the original samples tested for mutagenicity.

Butylene oxide was not identified in either sample tested by Snow et al. (1979), yet this mutagen was present in 19 of 22 samples analyzed by Henschler et al. (1980). It is possible that this substance was present in the original samples tested by Snow et al. (1979) but has since degraded. However, if the chemical analyses reflect the original composition of the material tested for mutagenicity and butylene oxide was not present, it is likely that the positive responses reported by Snow et al. (1979) were due to MC itself. Vinylidene chloride and trichloroethylene were present only as minor components, and most of the impurities were accounted for by acetone, methyl ethylketone, toluene, and dioxane. Even if one were to assume that the 3% impurities consisted exclusively of vinylidene chloride or trichloroethylene, it is not likely that the mutagenic responses can be accounted for by these substances. Both substances require the presence of an exogenously supplied metabolic activation system to yield a positive response in bacteria, yet linear dose-related positive responses were obtained for the methyl chloroform samples without metabolic activation. Thus, the positive responses obtained by Snow et al. (1979) must have been due to MC *per se* or to a mutagenic stabilizer that degraded between the time of testing and that of chemical analysis.

Nestmann et al. (1980) used *Salmonella* strains TA98, TA100, and TA1535 both with and without PCB-induced rat liver S9 mix in their tests of MC.

Aliquots of 100, 500, and 1000 µl (Fisher Scientific; purity not given) were placed in open glass dishes inside a dessicator. The plates were exposed to MC vapors for 16 hours and incubated for a total of 72 hours before scoring. The authors report that under the conditions of the test, MC was not detected as mutagenic by strain TA98 but induced up to a 2.5-fold increase in numbers of revertants in strain TA100 and up to a 6.5-fold increase in revertants in strain TA1535. However, the authors' conclusions cannot be independently assessed, because experimental values were not presented.

Gocke et al. (1981) used Salmonella strains TA100 and TA1535 both with and without PCB-induced rat liver S9 mix for metabolic activation to assess the mutagenic potential of MC (Merck Darmstadt, purity not given). Aliquots of 500, 1000, 1500, and 2000 µl (values extrapolated from a figure in the paper) were placed in airtight desiccators, and the bacteria were exposed for 8 hours. After incubation and scoring, a 1.3-fold increase in revertants was recorded for TA100 and a 10-fold increase in revertants was recorded for TA1535 at the highest dosage.

The results of Snow et al. (1979), Nestmann et al. (1980), and Gocke et al. (1981) strengthen the claim by Simmon et al. (1977) that commercial samples of MC are weakly mutagenic in Salmonella when exposure occurs in sealed chambers. The nearly identical responses obtained with samples of MC obtained from different sources is consistent with the hypothesis that MC itself is a weak mutagen in Salmonella, causing primarily base-pair substitution mutations. However, firm conclusions cannot be reached because of the possibility that the mutagenic activity is ascribable to chemical substances added as stabilizers or to impurities.

For proprietary reasons, manufacturers of MC are reluctant to divulge the identity and amount of chemical substances they use as stabilizers in their commercial products. Without this knowledge, however, it is not possible to determine whether the mutagenicity of these products is due to MC or to its stabilizers.

Commercially available samples of MC are clearly weakly mutagenic in Salmonella. Two studies, both unpublished, have been conducted using MC samples with known levels of identified mutagenic stabilizers (Domoradzki, 1980 and Williams and Shimada, 1983). The argument is made that the activity of commercial grade MC is due to the presence of highly mutagenic materials, such as butylene oxide, which are present as stabilizers.

In the study by Domoradzki (1980) CHLOROTHENE VG Solvent* (i.e., 93.33% MC, 0.73% 1,2-butylene oxide, and 5.94% other stabilizer components), low stabilized MC (i.e., 99.9%+ MC and 0.038% 1,2-butylene oxide), and 1,2-butylene oxide (i.e., 99+% 1,2-butylene oxide, < 0.2% butyraldehyde, and < 1% iso-butylene oxide) were evaluated for mutagenic activity in the Salmonella/mammalian microsome mutagenicity assay. Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to the test substances in airtight desiccator jars with and without Aroclor 1254-induced rat liver S9 mix. The vapor pressures of the test chemicals at 30°C were determined from Antoine constants, and the concentrations of the test materials were calculated using the ideal gas law. Test plates containing the indicator bacteria were placed in the desiccators, exposed to the test material for 60 hours, and subsequently scored. Positive and negative controls were performed concurrently with the test chemicals. No monitoring of the actual exposure concentrations was performed.

Positive responses (approximately tenfold increases over the spontaneous level) were obtained in strain TA1535 for CHLOROTHENE VG Solvent (at 20,664 and 41,328 ppm methyl chloroform, or 2.1 and 4.1%, respectively) and 1,2-butylene oxide (from 39 to 1198 ppm or 0.001 to 0.12%) and in strain TA100 for 1,2-butylene oxide (from 599 to 1198 ppm or 0.06 to 0.12%) both with and without metabolic activation. No increase in revertants over the spontaneous level was observed when low stabilized methyl chloroform was tested in these strains (Domoradzki, 1980).

Williams and Shimada (1983) also tested fully stabilized (Triethane 324A) and low stabilized (Triethane 321, 99.80% MC) samples of MC for mutagenicity in the Salmonella/mammalian microsome assay. The substances were obtained from PPG Industries' Lake Charles Plant. Purity analyses were performed by the Barberton Technical Center, but the percent composition of MC in the fully stabilized material was not given, and the identities and amounts of stabilizing materials were not reported for proprietary reasons. Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to 0, 2.5, 5.0, 7.5, or 10% MC for 18 hours in sealed chambers and then incubated for an additional 30-54 hours. Gas chromatographic analysis of MC in the exposure chamber was performed for one dose and showed that the desired air concentration of the test material for the dose was reached within the limits of sampling error

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*Trademark of Dow Chemical Company.

(i.e., for the 5% dose level, 5% MC was measured at 0 hours, and 4.2% was measured at 17 hours).

Treatment with low stabilized MC led to revertant counts twofold above background for strains TA100 and TA1535 with and without metabolic activation (i.e., Aroclor-induced rat liver S9 mix), but only at the 10% concentration level (100,000 ppm) of MC. This concentration resulted in greater than 95% toxicity. Fully stabilized MC caused increases in numbers of revertant colonies in TA1535 at concentrations of 2.5% (25,000 ppm) and higher with and without metabolic activation; increased numbers of revertants in TA100 were detected at 2.5% (25,000 ppm) with activation and at 5% (50,000 ppm) without activation (Williams and Shimada, 1983).

The results of Domoradzki (1980) and Williams and Shimada (1983) show that commercially available samples of MC are mutagenic. They are consistent with the hypothesis that the mutagenic activity is due to the presence of mutagenic stabilizers, but they do not prove the hypothesis. In summary, commercial samples of MC are weakly mutagenic to Salmonella TA1535 and TA100. It may be that the mutagenic response is caused by chemical substances added as stabilizers or to contaminants of manufacture.

5.3.2.2 Gene Mutations in Eukaryotes--In addition to testing MC in Salmonella, Gocke et al. (1981) used the Drosophila sex-linked recessive lethal test (Table 5-12). A 25 mM solution of MC in 5% saccharose was fed to wild-type Berlin K male flies for an unreported period of time (25 mM is reportedly close to the LD_{50}). These males were then mated to Basc females. Three broods were scored (i.e., offspring from virgin females mated to treated males on days 1-3, 4-6, and 7-10 after exposure). Two lethals were observed in 1226 cultures screened in the first brood; the values for broods 2 and 3 were 0/557 and 0/76, respectively. These values do not demonstrate an increased incidence in sex-linked recessive lethals. However, the experiment is an inadequate test, because the small number of chromosomes scored would preclude the detection of a moderate or weak mutagen. At least 7000 chromosomes must be scored in order to make the determination that exposure to a chemical does not increase the mutation frequency twofold (Lee et al., in press). In addition, the actual amount of MC ingested by the exposed males in the tests by Gocke et al. (1981) may have been less than predicted, because of the volatility of the substance.

Loprieno et al. (1979) reported that pure, unstabilized MC (Dr. Silvio Paglialunga, Coordinamento Medicina Ambiente Tossicologia Industriale, personal

TABLE 5-12. MUTAGENICITY TESTING OF METHYL CHLOROFORM: GENE MUTATIONS IN EUKARYOTES

Reference	Test System	Genetic Cross	Dose	Results			Comment
				Brood			
				I	II	III	
Gocke et al. 1981	*Drosophila* sex-linked recessive lethals	*Basc* x *Berlin K*	25 mM in 5% saccharose	2/1226	0/557	0/76	1. Small sample size precludes detection of moderate and weak mutagens. At least 7000 chromosomes should be scored to detect twofold increase in mutation frequency. 2. MC may have evaporated from saccharose solution. 3. Broods correspond to days 1-3, 4-6, and 7-10 posttreatment, respectively.

Reference	Test System	Strain	Activation System	Concentration	Results		Comment
Loprieno et al. 1979	*Schizosaccharomyces pombe* forward mutation	ade 6-60/ rad 10-198/ h-	Host-mediated assay in B6C3F1 mice	0 or 5000 mg/kg administered by gavage to mice. Yeast exposed for 3, 6, or 16 h.			1. Preliminary results; protocol not fully presented. 2. MTD may not have been employed. 3. Unclear if appropriate controls performed concurrently. 4. Inconclusive results because insufficient sample size screened and uncertainty regarding exposure of test organisms.
				Treatment Time (hours)	Methyl Chloroform (mg/kg)		
					0	5000	
				3	9/118386 (0.008%)	2/82814 (0.002%)	
				6	8/80552 (0.010%)	9/115831 (0.008%)	
				16	11/87183 (0.013%)	16/165588 (0.010%)	

communication, 1981), administered to B6C3F1 hybrid mice by gavage (5000 mg/kg), did not increase the incidence of forward mutations in Schizosaccharomyces pombe in a host-mediated assay. The yeasts were injected intraperitoneally, and measurements were made after treatment times of 3, 6, and 16 hours. The control values (mutations/cells scored) for the 3 time points were 9/118,386, 8/80,552, and 11/87,183, respectively. The corresponding values for the treated cells were 2/82,814, 9/115,831, and 16/165,588. In this forward mutation assay, which is based on the detection of altered colony color, there is no selection technique for detecting mutants. Therefore, the most important consideration in the analysis of this test is the number of colonies scored. No more than 1.65 x 10^5 cells were scored for any exposure period. Additionally, no information was provided concerning the ability of MC to be absorbed and transported to the peritoneum, thereby adequately exposing the yeast cells. Thus, the possibility of weak mutagenicity cannot be excluded because of the limited sample size, coupled with the possibility of limited exposure to the test organisms. The report also does not provide adequate information concerning the design of the tests, and this makes it difficult to assess the significance of the results.

5.3.2.3 Chromosomal Aberrations--Two studies have been published on the ability of MC to cause chromosomal aberrations (Table 5-13). In the test by Quast et al. (1978) Sprague-Dawley rats were exposed to 0, 875, and 1750 ppm MC for 6 hours/day, 5 days/week, for 52 weeks. The highest dose was about 1/8 the reported LD_{50}. Three males and three females per group were sacrified, and bone marrow cells were analyzed for aberrations. Fifty cells per male and fewer than 50 cells per female were scored. Although no increased incidence of aberrations was observed, the small number of cells analyzed makes the study inadequate. Also there was no concurrent positive control.

Gocke et al. (1981) assessed the ability of MC (Merck, Darmstadt; purity not given) to cause micronuclei in polychromatic erythrocytes (PCE). Two male and two female NMRI mice were used for each of 3 dose levels (266, 1000, and 2000 mg/kg). The highest dose approximates the LD_{50} for mice. Two intraperitoneal injections of each dose were given at 0 and 24 hours, and the animals were sacrificed at 30 hours; bone marrow smears were made and 1000 PCE per animal were scored for the presence of micronuclei. An apparent dose-related response was observed. The untreated controls had 1.3% micronuclei compared to 2.9% in the animals receiving 2 injections of 2000 mg/kg. Although these values represent a twofold increase in micronucleus formation, the control

TABLE 5-13. MUTAGENICITY TESTING OF METHYL CHLOROFORM: MAMMALIAN IN VIVO CYTOGENETICS TESTS

Reference	Test System	Strain	Sex	Concentration		Result	Comments
				ppm	# Cells	# Aberrations	
Quast et al. 1978	Chromosome aberrations in rat bone marrow cells	Sprague-Dawley, spartan substrain	Males	0	149	0	1. Three animals of each sex were exposed by inhalation 5 days/week, 6 h/day for one year. 2. Number of cells scored insufficient for adequate interpretation of the results. 3. Treatment had no effect on body weights of rats. MTD may not have been employed although dose used is about 1/8 the reported acute LD_{50}. 4. Judged to be inconclusive.
				875	150	0	
				1750	150	0	
			Females	0	105	0	
				875	65	1*	
				1750	38	0	
Gocke et al. 1981	Micronucleus formation in mouse bone marrow	NMRI		mg/kg	%Micronuclei		1. Two i.p. injections (one each at 0 and 24 h of 0, 266, 1000, and 2000 mg/kg). 2. Spontaneous control level is low compared to other control levels reported in paper (i.e, about 2%). Apparent twofold increase in micronucleus formation judged to be suggestive but not demonstrating positive response.
				0	1.3		
				266	2.0		
				1000	3.0		
				2000	2.9		

*Chromatid aberration from one animal from which 15 cells are analyzed.

value was low compared to the other controls reported in the paper (about 2% PCE with micronuclei). Thus, the response is considered to be merely suggestive of a positive effect.

5.3.2.4 Other Indicators of DNA Damage--Litton Bionetics (1975) tested MC for its recombinogenicity, as indicated by mitotic gene conversion in the yeast *Saccharomyces cerevisiae* (Table 5-14). The study was conducted at the same time as the testing of MC in bacteria, and many of the deficiencies noted above concerning the bacterial tests apply to the yeast tests as well (e.g., no special precautions were taken to prevent evaporation of the test compound; the compound was not soluble under the conditions of the test; and the toxicity results were highly variable). The results were negative, but the tests are judged to be inadequate. The volatility and insolubility of MC and the absence of reproducible, dose-dependent toxicity suggest that the cells may not have been adequately exposed.

In unpublished papers, Williams and Shimada (1983) and Williams (1983) tested samples of MC samples for their ability to cause unscheduled DNA synthesis in rat and mouse primary hepatocyte cultures (HPC DNA repair assay). In the Williams and Shimada (1983) report two samples were tested using rat primary hepatocytes (Table 5-14). One was fully stabilized and contained 0.02% vinylidene chloride, 0.35% butylene oxide, 5.3% unidentified materials, and 94.1% MC. The other sample, referred to as "low-stabilized," contained 0.02% vinylidene chloride, 0.1-0.2% unidentified materials, and 99.80% MC. Two types of test were conducted. One was a conventional HPC assay, in which the hepatocytes were exposed to MC added to the culture medium. The other test was a modified HPC assay, and the cells were placed in small glass dishes in a sealed incubator and exposed to MC gas. Tritiated thymidine (^{3}H-TdR) was added to the culture medium in both types of tests. If MC caused DNA damage repaired by the excision repair pathway, ^{3}H-TdR would be incorporated into the repaired DNA. To detect such incorporation, the cells were fixed on glass slides and subsequently coated with radiotrack emulsion. After an incubation period, the slides were developed and examined microscopically to count developed silver grains in the radiotrack emulsion over the cell nuclei.

Williams and Shimada (1983), used 2-acetylaminofluorene and vinyl chloride as positive controls for the conventional assay and the modified gaseous exposure assay, respectively. 2-acetylaminofluorene was highly active (170 grains/nucleus at 10^{-5}M) and vinyl chloride was weakly active at 2.5 and 5% (5 and 11

TABLE 5-14. OTHER TESTS OF THE GENOTOXIC POTENTIAL OF METHYL CHLOROFORM

Reference	Test System	Strain	Activation System	Concentration Formulation	Concentration %	Result	Comment
Litton 1975	Saccharomyces cerevisiae: gene conversion	D4	PCB-induced Sprague-Dawley rat liver S9 mix.	99+	4.5 2.25	Inconclusive	1. All formulations reported to be nonsoluble at the test concentrations employed. 2. Toxicity reported to be variable (in many cases as many or more survivors observed at the high dose as at the low dose). 3. No information about stabilizers or other chemical substances in the formulations. 4. No special precautions taken to prevent evaporation of MC, but tests reported to be conducted in suspension.
				96	2.0 1.0		
				95.65	2.0 1.0		
				93.75	5.0 2.5		

	Test System	Test Chemical	Concentration %	3-hr Exposure Time Autoradiograph Grains/Nucleus	3-hr Exposure Time Cytotoxicity	18-hr Exposure Time Autoradiograph Grains/Nucleus	18-hr Exposure Time Cytotoxicity	
Williams and Shimada 1983	Primary rat hepatocyte culture DNA repair assay	Vinyl chloride	5	Not done	Not done	11.3 ± 1.9*	0	1. Results from modified, gas exposure assay presented here. 2. Test is inconclusive because of questions about sensitivity. Positive control yielded only a weak response. Data on cytoplasmic grain count not presented; if high, system would only be able to detect agents causing large increases in unscheduled DNA synthesis. 3. Cytotoxicity identified by the absence of S-phase cells and general morphology.
			2.5	Not done	Not done	4.9 ± 3.5	0	
		Methyl chloroform (stabilized)	5	1.3 ± 5.8	4+	Not done	Not done	
			2.5	1.1 ± 2.8	3+	Not countable	4+	
			1	0	2+	0.84 ± 0.7	1+	
			0.1	0	0	0.76 ± 0.4	1+	
		Methyl chloroform (low stabilized)	2.5	Not countable	4+	2.3 ± 2.0	1+	
			1	0.6 ± 1.7	4+	4.6 ± 3.8	0	
			0.1	0.9 ± 2.2	3+	3.1 ± 0.9	0	
		Cell control		0	0	2.75 ± 2.76	0	

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*Grain counts from 20 nuclei.

TABLE 5-14. (continued)

Reference	Test System	Strain	Test Chemical	Concentration	Autoradiograph Grains/Nucleus	Cytotoxicity	Comment
Williams 1983	Primary hepatocyte culture DNA repair assay	Mouse/ $B_6C_3F_2$	Methyl chloroform	%			1. Source and purity not reported. 2. Data presented as net grain counts (i.e., background counts are subtracted). 3. Cytotoxicity identified by the absence of S-phase cells and general morphology. No indication given for toxicity in rat cells. 4. Inverse dose-response may be due to toxicity. 5. All doses at which the rat cells were tested may have been too high. 6. Positive response in mouse cells. Inconclusive in rat cells.
				1	2.6 ± 6.5	Minimally toxic	
				10^{-1}	Toxic	Toxic	
				10^{-2}	7.9 ± 14.7	Minimally toxic	
				10^{-3}	3.0 ± 4.8	Minimally toxic	
				10^{-4}	41.8	Non-toxic	
				10^{-5}	63.1 ± 59.1	Non-toxic	
				10^{-6}	74.5 ± 55.7	Non-toxic	
			Benz(a)pyrene	10^{-2}M	130.5 ± 41.3		
				10^{-4}M	42.1 ± 40.4		
			DMSO		2.3 ± 5.4		
		Rat/Osborne Mendel	Methyl chloroform	1	2.7 ± 4.17		
				10^{-1}	0.5 ± 1.7		
				10^{-2}	0.5 ± 0.87		
				10^{-3}	1.4 ± 2.3		
				10^{-4}	1.5 ± 2.7		
			Benz(a)pyrene	10^{-4}M	121.4 ± 19.0		
			DMSO		0.4 ± 0.69		

*Grain counts from 20 nuclei.

grains/nucleus, respectively) in increasing unscheduled DNA synthesis compared to the negative controls (3 grains/nucleus).

In the modified HPC DNA repair assay, cells were exposed to dosages up to 5% MC in the air for 3 or 18 hours. No increase in the grain count was observed (the number of grains/nucleus ranged from 0.6 ± 17 to 4.6 ± 3.8 in treated cells compared to 2.75 ± 2.76 in the untreated controls). Similarly, negative results were reported in the conventional assay where the cells were exposed to concentrations as high as 1% MC in the medium.

There are several factors to consider in the analysis of this assay. (1) The system is designed to detect the occurrence of a specific type of repair (i.e. "long-patch" excision repair). Xenobiotics, which cause damage that is not repaired or that is repaired by another mechanism, will not be detected as possessing genotoxic activity in the system. (2) The endpoint measured may not actually reflect repair synthesis of chromosomal DNA in the nucleus (Lonati-Galligani et al., 1983). Enhanced incorporation of 3H-TdR into mitochondrial DNA or suppressed ^{3}H-TdR incorporation into mitochondrial DNA without a concomitant increased or decreased incorporation into nuclear DNA can lead to false negative or positive results, respectively. Thus, both nuclear incorporation and cytoplasmic incorporation should be measured and the dose-responses plotted for each to serve as a basis for deciding whether or not the compound has induced UDS. (3) Nuclear grain counts vary with nuclear size (Lonati-Galligani et al., 1983); thus, it may be appropriate to express grain counts as a percentage of the measured nuclear area. In the studies by Williams and Shimada (1983) the values were transformed by subtracting cytoplasmic grain counts from total nuclear grain counts to give net grain count; as a result, the data are difficult to evaluate, and the way they are presented may preclude the detection of weakly active agents. Thus, although the results of this study are negative, they do not provide convincing evidence that MC does not induce UDS.

In the Williams (1983) study, MC (source and purity not reported) was assayed in the HPC DNA repair test using a conventional liquid exposure protocol. Primary hepatocytes from $B_6C_3F_2$ mice and Osborne Mendel rats were tested in two separate test series. A positive response, apparently inversely proportional to dose, was obtained in the mouse cells between 10^{-6}M and 10^{-4}M (a greater than 30-fold increase in grain count was noted at 10^{-6}M MC compared to the negative controls). The inverted shape of the dose-response curve may be due to toxicity. MC was reported to be nontoxic between 10^{-6}M and 10^{-4}M, but the criteria for measuring toxicity (i.e., absence of S-phase cells and general morphology) is subjective and perhaps not sensitive. It would have been preferable if lower doses had been used to determine the minimal effective dose and to determine a range of concentrations yielding positive dose-response. A negative response was obtained in testing conducted with rat cells (see Table 5-14) but the lowest dose tested was 10^{-4}M MC. At this dose, the response in the mouse cells was suboptimal. It would be appropriate to test lower doses using rat hepatocytes to rule out the possibility that a positive response would have been observed had a lower dose been used. Positive and negative controls were employed for both sets of experiments and responded appropriately, but the concerns regarding the presentation of data in the Williams and Shimada (1983) paper apply here as well. In spite of this, the positive response in the mouse cells shows that a commercially available sample of MC is genotoxic in mammalian cells. It should be noted that the positive response may be due to stabilizer materials present in the sample tested.

It is noteworthy that MC was one of the compounds tested in the collaborative study entitled the International Program for the Evaluation of Short-Term Tests for Carcinogenicity (IPESTTC) (de Serres and Ashby 1981), which was sponsored by Imperial Chemical Industries and the Medical Research Council in the United Kingdom and the National Institute of Environmental Health Sciences in the U.S.A. The study assessed the ability of short-term tests to predict the carcinogenicity of 42 coded chemical substances of high purity in 23 different assays (e.g., gene mutation tests in bacteria, yeast, and mammalian cells in culture; SCE formation _in vitro_ and _in vivo_; chromosome aberrations _in vitro_, etc.). Overall, the results were negative. Although a battery of tests was conducted in this study, it is judged not to provide an adequate assessment of the mutagenicity of MC. The intent of the program was to determine how well standard assays compared when testing coded samples. Many of the chemical substances selected for testing were chosen because of the difficulty of detecting them in short-term assays. Preliminary toxicity and range-finding tests were limited, because only small samples of the test materials were sent to the investigators. Furthermore, no information was provided to the investigators about the solubility and volatility of the materials, and the investigators were encouraged to use standard protocols. Thus, volatile and insoluble compounds like MC would not be likely to give positive responses, especially if they are weak mutagens.

5.3.2.5 Gonadal Effects--There are two studies that bear on the ability of MC to reach germinal tissue and cause adverse effects. In the study by Riddle et al. (1981), male and female ICR Swiss mice were given daily doses of 0, 99.4, 2,640, or 8,250 mg/kg in their drinking water. It was reported that the oral LD_{50} was about 10,000 mg/kg. No dose-dependent effects on fertility, gestation, viability, or lactation indices were observed, and no dose-related effects were detected in the F/0 generation. These conclusions cannot be evaluated, however, because they were presented in abstract form without supportive data.

Topham (1980) gave five daily i.p. injections of MC (purified by J. Ashby of Central Toxicology Laboratory of ICI Ltd., Alderly Park, U.K.) to groups of five (CBA x BALB/c) F_1 male mice. The doses were 0.1, 0.25, 0.5, 1.0, and 2.0 mg/kg. The highest dose was reported to be about half the LD_{50}; however, this is not consistent with other investigators who reported the LD_{50} for i.p. injection of MC in mice to be from 2 to 16 g/kg (e.g., Gocke et al., 1981). Five weeks after the last dose, smears of caudal sperm were coded and examined for head abnormalities. Cyclophosphamide monohydrate (20 mg/kg/day/5 days) and injections of either saline, corn oil, or 0.5% Tween 80 in water were used as concurrent positive and vehicle controls, respectively. MC was reported to be negative in this study, but the conclusions cannot be confirmed because no data were presented. Therefore, conclusions cannot be drawn concerning the ability of methyl chloroform to reach germ tissue because the information is too limited.

5.3.2.6 Summary and Conclusions--Methyl chloroform has been tested for its ability to cause gene mutations in *Salmonella* and *Drosophila*, chromosomal mutations in rats and mice, and unscheduled DNA synthesis in rat hepatocytes. It has also been tested for its ability to cause adverse effects in mouse testes, as indicated by the dominant lethal test and the sperm abnormality test. In addition, it was one of 42 chemicals tested in a battery of mutagenicity assays (e.g., gene mutation tests in bacteria, yeast, mammalian cells in culture; SCE formation *in vitro* and *in vivo*; chromosome aberrations *in vitro*, etc.) as part of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity (IPESTTC). However, these tests and most of the other experiments are judged to be inadequate because conventional protocols were used; MC is difficult to test under such conditions because of its volatility and relatively low solubility in water. Care must be taken to ensure that the indicator organisms (or appropriate cells in whole animal tests) are exposed to the materials in sufficient concentrations to provide an adequate test.

Commercially available MC has been shown to be weakly mutagenic in Salmonella, under special treatment conditions, by several different laboratories. Negative responses have been reported in gene mutation tests in yeast and in the Drosophila sex-linked recessive lethal test. However, these negative results cannot be interpreted as indicating a lack of mutagenic potential of MC, because no precautions were taken to ensure adequate exposure of the test organisms or because of such factors as small test populations or uncertainties about the chemical composition of the material tested. Chromosomal aberration tests have been reported to be negative, but these tests are also inadequate because of inadequacies in experimental design, such as the failure to score a sufficiently large experimental population. It should be noted that a micronucleus test yielded an equivocal weak positive response, but this apparent effect may be due to the low concurrent negative control values. Although MC has not been shown to be mutagenic in a higher eukaryote, a positive response was obtained in an unscheduled DNA synthesis assay using cultured primary hepatocytes from mice. This test indicates the potential of MC for causing genetic damage in mammalian cells.

The ability of MC to reach the gonads and cause genetic damage has not been systematically studied. Only two studies were identified--a dominant lethal assay and a test for morphologically abnormal sperm heads. Both reported negative results but do not provide convincing evidence that MC has no potential to cause heritable effects. The dominant lethal assay is not a sensitive test and the assay for morphologically abnormal sperm heads is not characterized genetically.

Based on the weight of available evidence, it is concluded that commercially available samples of MC are genotoxic to mouse hepatocytes and are weakly mutagenic in Salmonella under treatment conditions where sufficient exposure is ensured. The available data are inadequate, however, for reaching firm conclusions regarding the ability of MC to cause gene mutations in other organisms; however, the possibility that this substance, its associated stabilizing materials, or its metabolites may have mutagenic effects in humans has not been eliminated.

In view of the deficiencies in the available information, additional testing is advisable. At least three additional assays should be conducted to resolve whether commercially available samples of MC are mutagenic in organisms other than bacteria. These assays should include a test for gene mutations in a nonbacterial system (e.g., mammalian cells in culture), a test for chromosomal aberrations (e.g., an *in vivo* or *in vitro* mammalian test), and a test for other indications of DNA damage (e.g., sister chromatid exchange or DNA binding). Experiments are needed to determine whether methyl chloroform is mutagenic in the absence of stabilizing materials or contaminants. An approach with the potential to resolve this issue is to fractionate a mutagenic sample of MC and subsequently demonstrate whether the mutagenic activity is associated with the MC peak. Of course, in order to resolve the issue clearly, the fractionation would have to be performed in such a manner as to separate the components of the commercial sample without chemical modification. Even if it is shown that the mutagenic activity of MC is due to one or more of the stabilizers, it would be appropriate to ensure that commercial formulations of MC are also adequately tested, because human exposures generally involve the commercial formulations rather than purified samples.

Because of MC's physical properties, all additional studies should be designed to ensure appropriate exposure of the indicator organisms; specifically, precautions should be taken to prevent excessive evaporation and to overcome the low solubility of the compound in water.

5.3.3 Evaluation of the Carcinogenicity of Methyl Chloroform

The purpose of this section is to provide an evaluation of the likelihood that MC is a human carcinogen. The evalution of carcinogenicity depends heavily on animal bioassays and epidemiologic studies when available. However, information on mutagenicity, pharmacokinetic behavior, and metabolism, particularly in relation to interaction with DNA, have an important bearing on the qualitative assessment of carcinogenicity. The available information on these subjects is reviewed in the other sections of this document. This section presents an evaluation of animal bioassays dealing with the carcinogenicity of MC and its contaminant, 1,4-dioxane, two cell transformation studies, and finally, a summary and conclusions dealing with all relevant aspects of carcinogenicity.

5.3.3.1 Epidemiologic Studies--No epidemiologic studies relating to the carcinogenicity of MC are available.

5.3.3.2 Animal Bioassays - Rats--Three carcinogen bioassays have been completed in rats: (1) a National Cancer Institute gavage study in Osborne-Mendel rats published in 1977; (2) a Dow Chemical Company inhalation study in Sprague-Dawley rats published in 1978; and (3) a National Toxicology Program (NTP) gavage study in F344/N rats described in a peer-reviewed draft report in 1983 (it has since been withheld for re-review by NTP for possible serious data discrepancies and, as such, is not further discussed here).

5.3.3.2.1 National Cancer Institute (1977) Rat Study--The National Cancer Institute (NCI) bioassay (1977) was done with technical grade MC purchased from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. The purity was checked by Hazelton Laboratories of America, Inc., Vienna, Virginia, using gas-liquid chromatography (GLC) and infrared spectrophotometry. Analyses by GLC showed that it contained 95% MC and 3% 1,4-dioxane, an inhibitor routinely added to commercial preparations of MC. The remaining 2% of the GLC peak area contained several minor impurities, two of which may have been 1,1-dichloroethane and 1,1-dichloroethylene. In this study, Osborne-Mendel rats were treated with 750 mg/kg and 1500 mg/kg MC in corn oil five times a week for 78 weeks by gavage (NCI 1977). The rats were observed an additional 32 weeks with the experiment ending at 110 weeks. Both males and females were used, with 20 of each sex being untreated controls and 50 of each sex being tested at each MC dose level.

The study was inadequate because only 3% of the treated rats survived to the end of the experiment. The survival of both sexes of dosed rats was less than that of the matched control groups, which was also inadequate in the males. In male rats, 6/20 (30%) of the controls, 32/50 (64%) of the low-dose group, and 36/50 (72%) of the high-dose group died within a year of the start of the study. The Tarone statistical test of survival showed a dose-related positive trend ($P < 0.001$) in the proportions of deaths over the period of the experiment, although this differential mortality is not reflected in the survival at 78 weeks. In female rats, 1/20 (5%) of the matched controls, 24/50 (48%) of the low-dose group, and 21/50 (42%) of the high-dose group died in the first year. As in male rats, the statistical test for a positive dose-related trend was significant ($P < 0.04$). In both sexes, the early mortality in the treated rats may have reduced the incidence of late-appearing tumors; this is especially true in the males, since none survived to the scheduled termination of the study.

Fewer of the rats receiving MC survived at both 78 and 110 weeks than did the positive control rats receiving the known carcinogen carbon tetrachloride

(Table 5-15). A variety of neoplasms (Table 5-16) were observed in the MC-treated groups, but with incidences not statistically different from matched controls. Because of the high mortality in rats, this negative result cannot be used to draw any conclusions regarding the carcinogenicity of MC. The NCI Clearing House on Environmental Carcinogens concluded that the carcinogenicity could not be determined from this study (NCI Clearing House 1977). It should be noted that the isomer, 1,1,2-trichloroethane, is carcinogenic in mice, inducing liver cancer and pheochromocytomas in both sexes. Dichloroethanes, tetrachloroethanes, and hexachloroethane also produce liver cancer in mice and other types of neoplasms in rats (Table 5-17).

5.3.3.2.2 The Dow Chemical Company (1978) Rat Study--The Dow Chemical Company study (Quast et al., 1978) treated groups of Sprague-Dawley rats by inhalation under conditions that were similar to those experienced by workers (6 hours/day, 5 days/week, over one-half of a lifetime). Rats were treated 12 months and observed until death or until they reached the age of 31 months. Doses of 875 and 1750 ppm were 2.5 and 5 times the threshold limit value of 350 ppm. The composition of the formulation of the MC is given in Table 5-18. The average frequency of tumor occurrence in the treated animals was similar to that of controls (Table 5-19).

When tumors at each site by tumor type, both benign and malignant, were examined, there were eight differences between control and treated animals at the $P < 0.05$ level (Fisher Exact Probability Test). Seven of these were decreased tumor incidence; one was an increase in ovarian granulosa cell tumors in females at the 875 ppm dose. There were no ovarian tumors in 189 controls; however, there were three in 33 female rats treated at 875 ppm ($P = 0.003$), and two in 82 female rats treated at 1750 ppm ($P = 0.14$). Since there was a smaller response at this site in the high-dose group than in the low-dose group, the positive findings may not be related to the administration of MC.

The Dow study suffers from two drawbacks: (a) the animals were treated for only 12 months rather than a lifetime, but they were observed for another 12 months, and (b) it is not evident that the maximum tolerated dose was used during the treatment period. There is no evidence that a range-finding study (subchronic) had been done before the start of the experiment. The treated animals in the Dow study did not differ from the untreated animals in body weight, terminal organ weight, or mortality. The only sign of toxicity was an increased incidence of focal hepatocellular alterations in female rats at the highest dosage.

TABLE 5-15. COMPARISON OF SURVIVAL OF CONTROL GROUPS, METHYL CHLOROFORM-TREATED AND CARBON TETRACHLORIDE-TREATED (POSITIVE CONTROL) RATS
(NCI Bioassay 1977)

	Methyl Chloroform			Carbon Tetrachloride		
Group	Initial No. of Animals	Number Alive at 78 Weeks	Number Alive at 110 Weeks*	Initial No. of Animals	Number Alive at 78 Weeks	Number Alive at 110 Weeks*
Males						
control	20	7	0	20	20	12
low dose	50	1	0	50	34	15
high dose	50	4	0	50	35	8
Females						
control	20	14	3	20	18	14
low dose	50	9	2	50	38	20
high dose	50	12	1	50	21	14

*Time in study at last weighing.

TABLE 5-16. STATISTICAL ANALYSES OF THE INCIDENCE OF TUMORS AT SPECIFIC SITES IN MATCHED CONTROLS AND METHYL CHLOROFORM-TREATED RATS (NCI Bioassay 1977)

	Males*			Females*		
Topography: Morphology	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Total Animals: All Tumors†	3/20	6/48	6/50	7/20	7/50	9/50
P Values§	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	72	28	50	58	64	56
Pituitary: Chromophobe						
Adenoma†	0/20	0/48	0/48	3/20	2/48	1/48
P Values§	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	---	---	---	---	---	---
Thyroid: Follicular-Cell						
Adenoma†	0/20	0/48	0/50	2/20	0/50	1/49
P Values§	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	---	---	---	---	---	---
Adrenal: Cortical						
Adenoma†	0/20	3/49	1/50	2/19	1/48	2/49
P Values§	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	---	28	106	85	99	106

*Low- and high-dose groups received methyl chloroform in corn oil by gavage 5 times per week in time-weighted average doses of 750 and 1,500 mg/kg body weight, respectively.

†Number of tumor-bearing animals/number of animals examined at site.

§Beneath the incidence of the matched controls is the probability level for the Armitage test for positive dose-related trend in proportions when it is below 0.10; otherwise, N.S. = not significant. Beneath the dosed group incidence is the probability level for the Fisher Exact (conditional) Test for comparison of that dosed group with the matched control group when it is below 0.10; otherwise, N.S. = not significant.

TABLE 5-17. SUMMARY OF NCI/NTP CHLOROETHANE BIOASSAY RESULTS AS OF 1982

Compound	Species/Sex	Tumor Site	Statistically Significant Tumors
Monochloroethane	No Testing Planned		
1,1-dichloroethane	Retesting recommended because initial results inconclusive		
1,2-dichloroethane	rats/female	mammary gland	adenocarcinomas
	rats/male	forestomach	squamous cell carcinoma
		circulatory system	hemangiocarcinomas
		subcutaneous tissue	fibromas
	mice/female	mammary gland	adenocarcinomas
		endometrium	stromal sarcomas
		lungs	adenomas
	mice/male	lungs	adenomas
1,1,1-trichloroethane	mice/female	liver	hepatocellular carcinomas
1,1,2-trichloroethane	mice/female	liver	hepatocellular carcinomas
	mice/male	liver	hepatocellular carcinomas
	mice/male and female	adrenal glands	pheochromocytomas
1,1,1,2-tetrachloroethane	Final report in preparation		
1,1,1,2-tetrachloroethane	mice/female	liver	hepatocellular carcinomas
	mice/male	liver	hepatocellular carcinomas
pentachloroethane	Final report in preparation		
hexachloroethane	mice/female	liver	hepatocellular carcinomas
	mice/male	liver	hepatocellular carcinomas

TABLE 5-18. COMPOSITION OF THE FORMULATION OF METHYL CHLOROFORM USED IN CHRONIC INHALATION STUDIES IN RATS
(Quast et al. 1978)

Compounds*	Liquid volume%	Calculated weight%
Methyl chloroform	94.71	95.88
Nitromethane	0.44	0.38
Butylene oxide	0.74	0.46
1,4-Dioxane	3.93	3.11

*Analysis of methyl chloroform Lot TA02013B by gas chromatography.

TABLE 5-19. AVERAGE FREQUENCY OF TUMOR OCCURRENCES IN RATS TREATED WITH METHYL CHLOROFORM
(Quast et al. 1978)

	Number of Animals		Total Neoplasms/Animal	
	Male	Female	Male	Female
Control	189	189	1.06	2.97
875 ppm	91	92	0.85	2.67
1750 ppm	93	93	1.11	3.23

5.3.3.3 Animal Bioassays - Mice--Two carcinogen bioassays have been completed in B6C3F1 mice: (1) the National Cancer Institute (NCI) study in 1977 and (2) the study of the National Toxicology Program (NTP), released as a preliminary report in 1983. However, since the NTP study is undergoing re-review by NTP for possible serious data discrepancies, only the NCI study will be discussed here.

5.3.3.3.1 NCI (1977) Mouse Study--In the NCI (1977) bioassay, B6C3F1 hybrid mice were used with 20 animals of each sex in the control group and 50 animals of each sex at each treatment dose. The time-weighted average doses were 2807 mg/kg and 5615 mg/kg. The mice were treated by gavage 5 days a week for 78 weeks and observed for another 12 weeks for a total of 90 weeks in the experiment.

Table 5-20 summarizes the survival of the animals at 78 and 90 weeks. It shows that only 25 to 40% of those treated with MC survived until the time of terminal sacrifice. The NCI concluded that this early mortality in mice receiving MC may have lowered the incidence of late-appearing tumors. In addition, the treated animals gained less weight than the controls.

TABLE 5-20. COMPARISON OF SURVIVAL OF CONTROL GROUPS,
METHYL CHLOROFORM-TREATED, AND CARBON TETRACHLORIDE-TREATED
(POSITIVE CONTROL) MICE
(NCI Bioassay 1977)

	Methyl Chloroform			Carbon Tetrachloride		
Group	Initial No. of Animals	Number Alive at 78 Weeks	Number Alive at 90 Weeks	Initial No. of Animals	Number Alive at 78 Weeks	Number Alive at 90 Weeks
Males						
control	20	6	2	20	13	7
low dose	50	21	15	50	11	0
high dose	50	14	11	50	2	1
Females						
control	20	12	11	20	18	17
low dose	50	28	23	50	10	0
high dose	50	14	13	50	3	1

A variety of neoplasms (Table 5-21) were observed in treated groups but with incidence not statistically different from matched controls. Because of the high mortality in mice, this negative result cannot be used to draw any conclusions regarding the carcinogenicity of MC.

5.3.3.4 Cell Transformation Studies--Price et al. (1978) exposed Fischer rat embryo cell cultures (F1706, subculture 108) to MC liquid at concentrations of $9.9 \times 10^{+1}$ and 9.9×10^{2} μM for 48 hours. MC was diluted with growth medium to yield the appropriate doses. The MC sample obtained from the Fisher Scientific Company was purportedly $\geq$ 99.9% pure, but a personal communication from Mr. Carlson of the Fisher Scientific Company revealed that the MC (which was supplied by Fisher to Dr. Price) was really the product of the Dow Chemical Company and was actually about 95% pure. The chemical composition of Dow's MC as reported by Quast et al. (1978) of the Dow Chemical Company is given in

TABLE 5-21. STATISTICAL ANALYSES OF THE INCIDENCE OF TUMORS AT SPECIFIC SITES IN MATCHED CONTROLS AND METHYL CHLOROFORM-TREATED MICE
(NCI Bioassay 1977)

	Males*			Females*		
Topography: Morphology	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Total Animals: All Tumors†	2/15	2/47	6/49	4/18	2/48	3/50
P Values§	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	80	89	50	80	54	26
Hematopoietic System:						
Malignant lymphoma†	2/15	0/47	2/49	3/18	1/48	0/50
P Values§	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	80	---	64	80	90	---
Liver: Hepatocellular Adenoma or carcinoma or neoplastic nodule†	0/15	0/47	4/49	0/18	0/48	0/50
P Values§	P = 0.035	N.S.	N.S.	N.S.	N.S.	N.S.
Weeks to first observed tumor	---	---	---	---	---	---

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*Low- and high-dose groups received methyl chloroform in corn oil by gavage 5 times per week in time-weighted average doses of 750 and 1,500 mg/kg body weight, respectively.

†Number of tumor-bearing animals/number of animals examined at site.

§Beneath the incidence of the matched controls is the probability level for the Armitage test for positive dose-related trend in proportions when it is below 0.10; otherwise, N.S. = not significant. Beneath the dosed group incidence is the probability level for the Fisher Exact (conditional) Test for comparison of that dosed group with the matched control group when it is below 0.10; otherwise, N.S. = not significant.

Table 5-18. The cells were grown in Eagles minimum essential medium in Earle's salts supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 100 μg penicillin, and 100 μg streptomycin per ml. Quadruplicate cultures of Fischer rat embryo cells (F 1706, subculture 108) at the stage of 50% confluence were treated for 48 hours with low (9.9×10 μM) and high (9.9×10^2 μM) doses of MC. These doses were determined in previous experiments to be only minimally toxic. After treatment, cells were cultured in growth medium alone at 37°C and observed for successive subcultures.

Transformation of cells was characterized by progressively growing foci composed of cells lacking contact inhibition and orientation. In the low- and high-dose cultures, transformation was first observed at 23 days (two subcultures after treatment) and 44 days (five subcultures after treatment). There was no transformation of cells grown in medium alone or in the presence of 1:1000 acetone concentration.

At the fourth subculture after treatment, the cells were plated on soft agar (50,000 cells per dish) and held for 4 weeks at 37°C in a humidified CO_2 incubator prior to staining. In the low- and high-dose cells, 52 and 55 macroscopic foci were observed, respectively.

At the fifth subculture, cells from the low-dose treatment were injected subcutaneously into newborn Fischer 344 rats. Sixty-eight days after injection, fibrosarcomas were observed in all of the eight animals treated. Cells grown in growth medium alone were not injected, but cells treated with 1:1000 acetone induced no local fibrosarcomas in 13 animals injected.

Hatch et al. (1983) exposed Syrian hamster embryo cells to gaseous MC and measured the chemically induced enhancement of cell transformation by adenovirus. The agent used, obtained from Aldrich Chemical Company, was said to be 97% pure and to have 3% 1,4-dioxane added as a stabilizer (manufacturer's specifications). A special gas flow chamber was constructed to ensure exposure to the agent. They found that a statistically significant ($P < 0.05$) enhancement of activity occurred at three of four dose levels tested. They also mentioned that MC from other sources, which had no 1,4-dioxane, was just as active, but did not publish data for these other preparations.

5.3.3.5 Carcinogenicity of 1,4-Dioxane--Methyl chloroform contains a small amount of stabilizing substances. The concentration of specific stabilizers that had been identified in various commercial MC products is shown below (Aviado 1977 and Detrex 1976, cited by Mazaleski 1979).

	Volume %
Nitromethane	0.4 - 1.8
Butylene oxide	0.4 - 0.8
1,4-Dioxane	2.5 - 3.5
Dioxolane	1.0 - 1.4
Methyl ethyl ketone	1.0 - 1.4
Toluene	1.0 - 1.4
2-Butyl alcohol	0.2 - 0.3
Isobutyl alcohol	1.0 - 1.4

Not all of these stabilizers are in every product, but the maximum total inhibitor package (combinations of stabilizers) appears to be between 7 and 8% by volume (Aviado 1977, cited by Mazaleski 1979).

Since 1,4-dioxane is a contaminant in MC (about 3%), the carcinogenicity of 1,4-dioxane has been studied extensively. The results of these studies are summarized in Table 5-22.

It should be noted that 1,4-dioxane causes liver and nasal tumors in more than one strain of rats and hepatocellular carcinomas in mice. Liver tumors have been induced by dioxane in both male and female rats, as well as in mice. These animal results, coupled with a reported finding of nasal carcinomas in furniture workers exposed to 1,4-dioxane (NCI 1978, p. 108), suggest that 1,4-dioxane is a potential human carcinogen. A detailed evaluation of the carcinogenicity of 1,4-dioxane is currently being prepared by the CAG.

5.3.3.6 Summary and Conclusions--No epidemiologic studies are available investigating the carcinogenicity of MC.

In animal tests, two rat studies have failed to produce positive results for carcinogenicity. The NCI (1977) gavage study in Osborne-Mendel rats could not be interpreted because of extremely poor survival. The Quast et al. (1978) inhalation study in Sprague-Dawley rats showed no carcinogenic response except for ovarian granulosa cell tumors in the low-dose group. This site was not affected in the other rat studies. However, the lack of toxic effects in the Quast et al. (1978) study indicates that the dose was too small for maximum sensitivity.

In B6C3F1 mice, only one study is available. The NCI (1977) study showed no response, although relatively high mortality limited its sensitivity.

Technical grade MC has been shown to be weakly mutagenic in *Salmonella* and to transform rat and Syrian hamster animal cells *in vitro*. Technical

TABLE 5-22. SUMMARY OF ANIMAL CARCINOGENICITY STUDIES FOR 1,4-DIOXANE

Species	Strain	Route, Frequency of Administration	Sex	Control	Dose	Tumor Type	Reference
Rats	Wistar	In drinking water for 63 weeks	M	Untreated	300 mg/day average	Hepatocellular carcinoma, renal transitional cell carcinoma, myeloid leukemia, and lymphosarcoma	Argus et al. 1965
Rats	Sprague-Dawley derived Charles River CD	In drinking water for 13 months	M	Untreated	0.75, 1.0, 1.40, and 1.80%	Hepatocellular carcinoma (dose-response) 4/30, 9/30 16/30, 23/30	Argus et al. 1973
Guinea pigs		In drinking water for 23 months	M	Untreated	588-635 g in 23 mo.	Gall bladder carcinoma, hepatoma, renal adenoma	Hoch-Ligeti and Argus 1970
Rats	Sprague-Dawley derived Charles River CD	In drinking water for 13 months	M	Untreated	0.75, 1.0, 1.40, and 1.80%	Squamous cell carcinoma (nasal cavity), hepatocellular carcinoma, and fibroma	Hoch-Ligeti et al. 1970
Mice	Swiss Webster	Applied to shaved skin of back 3 times/week for 60 weeks	M F	Acetone Acetone	Unspecified Unspecified	Carcinoma, subcutaneous tumor	King et al. 1973
Rats	Sherman	In drinking water for 2 years	M & F	Untreated	0.01, 0.1, and 1%	Hepatic tumors P = 0.00022 (all types) Hepatocellular carcinoma P = 0.00033 Nasal carcinoma P = 0.054 (these tumors are at the 1% level)	Kociba et al. 1974
Rats	Wistar	Inhalation 7 hrs daily 5 day/week	M & F	Filtered air	111 ppm	No hepatic or nasal carcinomas Reticulum cell sarcoma M 18/150 vs. 37/206 ($P < 0.08$) F 18/139 vs. 30/207 Found many other types of tumors but not significant	Torkelson et al. 1974
Rats	Osborne-Mendel	In drinking water for 110 weeks	M & F	Untreated	0.5 and 1.0%	Hepatocellular adenoma ($P = 0.001$) and squamous cell carcinoma of the nasal turbinates ($P = 0.008$) (both sexes)	NCI Bioassay 1978
Mice	B6C3F1	In drinking water for 90 weeks	M & F	Untreated	0.5 and 1.0%	Hepatocellular carcinoma ($P = 0.001$) (both sexes)	

grade MC contains about 3% of the stabilizing compound 1,4-dioxane, which shows evidence of being an animal carcinogen. The possibility that this contaminant is responsible for the responses observed has apparently been ruled out in the case of hamster embryo cells, but remains an open question for the Salmonella findings and the rat embryo cell responses.

In conclusion, there are negative carcinogenic results in rats and mice and the possibility of contamination as a cause of the weak response in the mutagenicity of MC.

If the criteria of the International Agency for Research on Cancer (IARC) were used, the overall weight of evidence would indicate that the overall evaluation of MC would be equivalent to the IARC group 3 category of chemicals, in which a chemical cannot be classified as to human carcinogenicity.

5.4 SUMMARY OF ADVERSE HEALTH EFFECTS AND LOWEST OBSERVED EFFECTS LEVELS

5.4.1 Inhalation Exposure

The high volatility of methyl chloroform (MC), the extensive use of this compound as an industrial solvent, and the early interest in MC as an anesthetic has resulted in many reports in the scientific literature of human exposure to MC. Human experimental studies using acute and subchronic exposures have been performed using a few clinical-level tests of motor and cognitive performance. In many cases, these studies were poorly documented and studied only a few subjects using non-conservative statistics, where statistics were used at all. No studies using sensitive tests of cognitive performance or tests of vigilance or sensory function were found. While information on human chronic inhalation exposure to MC is available from studies of occupationally exposed groups, there is uncertainty in these studies as to the exact extent of exposure; some studies are complicated by simultaneous exposure to other chemicals used in the workplace. Although these human studies have deficiencies which preclude any individual study from being used for human risk assessment, the combined information presented in these studies can be used to estimate approximate dose-response relationships which provide a relatively clear description of the toxic effects of MC. However, because no quantitive studies or sensitive dependent variables were studied, no definitive conclusions about the effects of human exposure of MC can be drawn at this time.

Studies in experimental animals are available for acute and subchronic exposure to MC, but the uncertainty of extrapolation from animals to humans

TABLE 5-23. HUMAN FATALITIES ASSOCIATED WITH METHYL CHLOROFORM

Case No.	Sex	Material or Product Inhaled	Condition of Lungs at Autopsy	1,1,1-Trichloroethane Concentrations		Remarks	Source
				Blood mg%	Air ppm^x		
1	F	Energine	passive congestion	72	102,900		Hall and Hine, 1966
2	M	1,1,1-trichloroethane	no autopsy	--	--	75,000 ppm in work space; collapsed after leaving work area	Kleinfeld and Feiner, 1966
3	M	"degreaser"	acute congestion and edema	30.0	42,900		Bonventre et al., 1977
4	M	Chlorothene®	heavy, edematous	13	18,600	possible aspiration, pneumonia	Hall and Hine, 1966
5	M	1,1,1-trichloroethane	heavy, edematous	12.0	17,000		Stahl et al., 1969
6	M	"	heavy, edematous	6.3*	9,000		Stahl et al., 1969
7	M	"	heavy, edematous	6.2	8,900		Stahl et al., 1969
8	M	"	heavy, edematous	6.0	8,600		Stahl et al., 1969
9	M	"	moderately edematous	6.0	8,600		Hatfield and Maykoski, 1970
10	M	"paint remover"	moderately edematous	4.7*	6,700		Stahl et al., 1969
11	F	"paint remover"	acute edema & congestion	2.0	2,900	ethanol conc. in blood was 0.04%	Caplan et al., 1976
12	M	1,1,1-trichloroethane	markedly edematous	0.15	200	possible aspiration, pneumonia	Stahl et al., 1969

^{x}Calculations based on assumption from Stewart et al., 1969, of one hour exposure correlating to 0.07 mg percent in blood per 100 ppm in air.

*Estimated from brain concentration.

makes this animal data useful only in a supportive role for human risk assessment.

5.4.1.1 Effects of Single Exposures--Lethal effects of acute exposure to MC have been reported after accidental exposure and abuse, with death resulting from CNS depression followed by respiratory and cardiac failure (Hall and Hine, 1966; Bas, 1970; Stahl et al., 1969; Bonventre et al., 1977; Klienfeld and Feiner, 1966; Hatfield and Maykoski, 1970; Caplan et al., 1976). From the levels of MC in the blood and brain of cadavers, estimates have been made of the concentration of MC present in the air during the time the victim was exposed. These estimates range from 200 to 102,900 ppm (1,080 to 555,660 mg/m^3), with the majority of values being between 6,700 and 18,600 ppm (36,180 and 100,440 mg/m^3) (Table 5-23). However, these estimates are extremely crude and probably low since it is not known to what degree the MC had dissipated from the victims' bodies prior to obtaining tissue samples. Also, some of the observed deaths which occurred at relatively low MC concentrations may have resulted from aspiration pneumonia prior to extensive involvement of the CNS. A clearer indication of the levels of MC needed to produce narcosis is available in the study of Dornette and Jones (1960) investigating the use of MC as an anesthetic in humans. Although this study gives an indication of the levels of MC which cause CNS depression, presumably a higher concentration would be needed to cause depression severe enough to result in respiratory or cardiac failure. From these data and the LC_{50} concentrations of 14,250 ppm (75,950 mg/m^3) obtained by Adams et al. (1950) for rats, it can be estimated that a single short-term exposure to as much as 5,000 ppm (27,000 mg/m^3) would not be expected to be lethal; exposure to higher concentrations and longer duration of exposure, however, could produce narcosis and possibly death.

The effects of exposure of human volunteers to MC for single short-term periods were reported for MC levels far below the levels which cause narcosis (Table 5-24). Stewart et al. (1961) exposed 7 subjects to vapors of MC which were increased from 0 to 2,600 ppm (0 to 14,040 mg/m^3) in increments of 15 minutes each. The progress of observed effects is presented in Table 5-1, with 6 of 7 subjects complaining of throat irritation at 1,900 ppm (10,260 mg/m^3). The progressive exposure method used in this study and the small number of subjects makes the results difficult to interpret. However, in a second experiment in which subjects were exposed to 900 ppm (4,860 mg/m^3) MC for periods of 20, 35, or 73 minutes, difficulty was observed in the performance

TABLE 5-24. NON-LETHAL EFFECTS OF METHYL CHLOROFORM ON HUMANS

Study	Dose/Species	Effect
Dornette & Jones, 1960	10,000-26,000 ppm	Induction of anesthesia
	6,000-22,500	Maintain light anesthesia Depressed BP during mod. anesthesia Tendency to develop ventricular arrhythmias during hypoxia (reversed by oxygenation)
Siebecker et al., 1960	Anesthesia	Little change in EEG-similar to halothane anesthesia
Stewart et al., 1961	0-2,650 ppm for 15 min	See Table 5-1
	500 ppm	No effects
	900-1000 ppm	See Table 5-2
Torkelson et al., 1958	900-1000 ppm for 75 min	Slight eye irritation and lightheadedness; Flanagan and Rombert test showed slight loss of coordination and equilibrium
	1,900 ppm for 5 min	Equilibrium disturbed, positive Romberg test
Torkelson et al., 1958 Stewart, 1968; 1971 Am. Ind. Hyg. Assoc.	Results of single exposures	See Table 5-7
Stewart et al., 1969	500 ppm 6.5-7 hr/day for 5 days	Mild subjective complaints; abnormal Romberg
Stewart et al., 1975	500 ppm	See Table 5-3
Gamborale and Hultengren, 1973	250, 350, 450, 550 ppm for 4-30 min periods	Change in manual dexterity and perceptual tests in exp. vs. control groups
Salvini et al., 1971	450 ppm for 2-4 hr periods	Decreased perceptive capability under stress, eye irritation
Maroni et al., 1977	110-990 ppm	No effects
Seki et al., 1975	4, 25, 28, 53 ppm	No effects
Kramer et al., 1978	11-838 ppm → x = 115	No effects, although SGPT and albumin were different in exposed vs. control group.
Stewart and Andrews, 1966 Stewart, 1971 Litt & Cohen, 1969		See Table 5-4

of the Romberg tests, and some lightheadedness was experienced. No effects were observed in subjects exposed to 500 ppm (2,700 mg/m^3) for MC for 78 or 186 minutes. In both of these experiments, commercial grade MC was used which contained the inhibitors dioxane (2.4-3.0%), butanol (0.12-0.3%) and small amounts of 1,2-dichloroethane. These compounds may have also had some effect on the subjects. In support of the above findings, Torkelson et al. (1958) obtained similar results with 2 groups of 4 subjects each exposed to 500 or 900 ppm (2,700 or 4,860 mg/m^3) of pure MC for 75 to 90 minutes. Subtle changes in perceptive capability were observed following exposure to 350 and 450 ppm (1,890 and 2,430 mg/m^3) MC by Gamborale and Hultengren (1973) and Salvini et al. (1971). However, these last two observations are questionable since the effects of menthol used to mask the odor of MC in the first study were not evaluated, and in the second study the control subjects were not matched for all intervening variables such as food and drinking habits. Since the above-mentioned studies were largely qualitative and since only gross dependent variables were observed, conclusions are tentative at best.

Although a precise dose-response gradient of effects produced by MC exposure cannot be derived, it is possible to establish a crude relationship between exposure and effects from a compilation of the human data available. A summary of the estimated dose-response relationships for acute effects of single short-term exposures is presented below:

>5,000 ppm	Onset of narcosis
1,900-2,650 ppm	Lightheadedness, irritation of the throat
1,000 ppm	Disturbance of equilibrium
350-500 ppm	Slight changes in perception, obvious odor
100 ppm	Apparent odor threshold

The approximate concentrations of MC which elicit a particular adverse effect have greater uncertainty at the two extremes, that is, at levels greater than 10,000 ppm (270,000 mg/m^3), and the no-observed-adverse-effect level

(NOEL)[*], 350-500 ppm (1,890 to 2,700 mg/m^3). This results from the inherent difficulties in obtaining exact exposure values in the cases of accidental overexposure to MC, and the difficulty in the acquisition of either quantitative or qualitative data on slight behavioral changes at the lower exposure levels. Acute overexposure to MC may be limited by the low odor threshold in humans of 100 ppm (540 mg/m^3). Others report that an obvious odor is present at 500 ppm (2,700 mg/m^3). It also should be noted that there are no reports of residual adverse effects from a single exposure to MC.

Major uncertainties about the dose-response relationship estimated for MC from the compilation of the available human reports derive from (1) the lack of information on the length of exposure in accidental exposure and (2) the short exposure duration (<2 hours) in most of the studies in which controlled exposures of human volunteers were used. Although inhaled MC is retained in the lungs in proportion to the concentration in the air, the time to reach pulmonary steady-state conditions is relatively long (4 hours). Since the maximum body burden from a given exposure to MC was probably not reached in any of these studies, it is likely that exposures for longer times would result in a greater body burden and more severe effects at a given concentration. In the studies of the inhalation toxicity of MC, there are insufficient data to predict the results of the increased body burden of MC obtained by extending the exposure until steady-state conditions are obtained. However, from pharmacokinetic data presented in Figure 4-1, it can be reasonably concluded that the exposure levels noted in the above dose-response table would not shift by more than 30 percent. It should be emphasized that these approximations are only crude estimates.

5.4.1.2 Effects of Intermittent or Prolonged Exposures

There is a substantial amount of information from animal studies on the effects of subchronic or chronic inhalation exposure to MC. There is also a lifetime chronic study in rats. As discussed below, the only experimental study of humans involved repeated exposure to MC 5 days per week for 3 weeks and is of such a short duration that no information can be extrapolated from this study as to the long-term effects of MC exposure (Stewart et al., 1969).

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[*] NOEL: is defined as the exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

Observations of occupationally exposed workers exposed up to 6 years at MC levels (TWA) ranging from 50 to 249 ppm (270 to 1,345 mg/m^3) showed no difference between exposed and comparable control populations.

There are, however, three subchronic experimental animal studies with exposures continuing up to 180 days which provide some information on the effects of repeated exposures to MC. The study of Torkelson et al. (1958), using rats, guinea pigs, rabbits, and monkeys, and the study of Eben and Kimmerle (1974), using rats, established a no observed effect level (NOEL) for subchronic exposure to MC for 7 to 8 hours a day, 5 days per week for 3 months at 500 and 440 ppm (2,700 and 2,376 mg/m^3), respectively. Similarly, a NOEL of 370 ppm (1,898 mg/m^3) was obtained by Prendergast et al. (1967) in a variety of species (15 guinea pigs, 3 squirrel monkeys, 3 New Zealand rabbits, and 2 beagle dogs) exposed continuously to MC for 6 weeks. In this study there were 3 deaths in the low exposure group (135 ppm; 729 mg/m^3), but these were attributed to lung infections. These three studies would indicate that subchronic exposure to MC in the range of 370 to 500 ppm (1,898 to 2,700 mg/m^3) produces no gross signs of toxicity. However, the report of McNutt et al. (1975) indicates that exposure below this range may alter some biochemical parameters in the liver and brain.

McNutt et al. (1975) observed mild to minimal alterations in the rough endoplasmic reticulum, detachment of polyribosomes, microbodies, and triglyceride droplets in the livers of mice exposed to 250 ppm of MC continuously for 24 weeks. At 1000 ppm (5,400 mg/m^3), the extent of these changes had increased and some individual hepatocyte necrosis was observed. Although it is unclear whether the early biochemical and histologic changes observed at exposures of 250 ppm (1,350 mg/m^3) adversely affected liver function, they may represent the first stages in the sequence of events which leads to the hepatocellular necrosis observed at 1000 ppm (5,400 mg/m^3). The NOEL of 350 to 500 ppm (1,890 to 2,700 mg/m^3) discussed previously may reflect the insensitivity of the parameters investigated in the other studies.

The only study involving multiple experimental exposures of humans was that of Stewart et al. (1975), in which 20 subjects were exposed to 500 ppm (2,700 mg/m^3) of MC for 7.5 hours a day, 5 days per week for 3 weeks. There were no effects on the clinical chemistry of the blood or urine, nor on pulmonary function tests. However, the female subjects did have an increased number of complaints of odor. The authors stated that there was a lack of sleepiness and fatigue which had been reported in a previous study. Again, these tests are not known to be sensitive enough to detect the slight changes in the liver observed in mice at exposures to 250 or 1000 ppm (1,350 or 5,400 mg/m^3) by McNutt et al. (1975). The combined evidence from the human and animal studies would indicate that only minimal effects are produced by continuous exposure to 250 ppm (1,350 mg/m^3) of MC and that the pathologic importance of these effects are unknown.

The studies of Maroni et al. (1977), Seki et al. (1975) and Kramer et al. (1978) reported a NOEL for workers occupationally exposed to MC levels as high as 350 ppm (1,890 mg/m^3). The short duration of exposure, averaging less than 1 year, of the work population in the report of Kramer et al. (1978) precludes the use of this study in human risk assessment. The study of Seki et al. (1975) provides information on 196 male workers exposed to MC for at least 5 years at concentrations of 4, 25, 28, and 53 ppm (22, 135, 151, and 286 mg/m^3). The number of workers exposed at each concentration was relatively small, with only 42 people exposed to the highest concentration. While no dose related effects were observed, the exposed groups were only compared among themselves and not with a control population. The reported details of the analytical procedures were insufficient to determine whether the analytical data represented measurements over the entire time period (5 years) of exposure of the study population, or whether the concentrations reported were the concentrations in the breathing zone. Most of the deficiences in this study would probably result in an underestimation of the exposure levels. Thus, it is difficult to ascertain any NOEL from this study. Maroni et al. (1977) looked for neurophysiological abnormalities in a population of 21 women who had been exposed for 6.5 years to an average concentration of MC between 110 and 345 ppm (594 and 1,863 mg/m^3). No neurotoxicity was observed in exposed workers, as compared to a control population of 7 unexposed women. The small size of the exposed and control populations, and the limited number of physiological measurements made makes the apparent NOEL from this study extremely uncertain.

5.4.2 Oral Exposure

There is little information on the toxicity of MC by the oral route. As summarized in Table 5-5, the acute LD_{50} for MC has been determined in rats, mice, guinea pigs, and rabbits by Torkelson et al. (1958) and the values range from 8,600 to 14,300 mg/kg body weight. These LD_{50} values could theoretically be used to calculate an approximate human lethal dose using the cubed root of the body weight ratios for interspecies conversion (U.S. EPA, 1980b; Freireich et al., 1966; Rall, 1969). However, the data obtained from the species studied in the report of Torkelson et al. (1958) do not show a relationship between MC toxicity and body weight. Therefore, without further information, it would not be justified to place much reliance on an approximate human lethal dose calculated using the above approach. However, as a crude indication of a lethal oral dose of MC, the values were calculated from each species with the range for a human lethal dose falling between 770 and 3940 mg of MC/kg. Also, there is no information from human exposure to MC to indicate whether values obtained from a calculated lethal dose are applicable to human toxicology. For the above reasons it is considered inappropriate to attempt to predict an approximate human lethal dose from the present data available.

There are subchronic studies on oral exposure to MC, and one chronic study. In the NCI (1977) bioassay, rats and mice were treated by gavage with MC in corn oil 5 days/week for 78 weeks at a level of 750 and 1500 mg/kg body weight for the low and high dose group rats, and 2807 and 5615 mg/kg body weight for the low and high dose group mice. Early deaths were observed in both rats and mice, with a statistically significant dose-related trend observed in male and female rats, and in female mice. The control animals also had poor survival and the early mortality in this group would suggest that MC contributed only partly to the early deaths of the treated animals. Another difficulty with this study is that treatment of animals by gavage is not identical to exposure through food or water since the daily dose of the compound is administered at a single time, resulting in high body levels immediately after treatment. As a result of the poor survival of the control animals and the inappropriateness of the route of administration, the relevance of this study to human risk assessment is questionable.

Both oral and inhalation studies have been performed to evaluate the teratogenic potential. While it is not possible to define the full potential of MC to produce human adverse teratogenic or reproductive effects, it does not appear that short- or long-term exposure, in studies performed to date, results in teratogenic effects in rats and mice.

5.4.3 Dermal Exposure

There is insufficient information available for quantitative risk assessment of dermal exposure to MC. The pharmacokinetic data of Fukabori et al.

(1967, 1977) would indicate that absorption of MC through intact skin can contribute significantly to the levels of MC in the body. The extent of dermal absorption was estimated to be 5 percent of respiratory absorption in humans in direct contact with the liquid. The high vapor pressure of MC would preclude dermal contact with this solvent for sufficient periods of time to allow toxic quantities to be absorbed, except in some industrial situations. Even so, the greater efficiency of absorption by inhalation would make this the route of greatest concern.

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